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Polimorfizm zimowego fenotypu w populacji heterotermicznego gryzonia, chomicznika dżungarskiego, Phodopus sungorus

Rozprawa doktorska

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Spis treści

1. Streszczenie

Polimorfizm to występowanie osobników o różnych fenotypach w obrębie populacji, wewnątrz której zachodzi swobodny przepływ genów. W środowiskach o wyraźnej sezonowej zmienności warunków otoczenia, zmiana fenotypu w cyklu życiowym umożliwia dopasowanie do aktualnie panujących warunków. W strefie klimatów umiarkowanych i okołobiegunowych zwierzęta zmieniające swój fenotyp na zimowy mają mniejsze wydatki energetyczne i powinny być faworyzowane przez dobór naturalny. Pomimo tego, nie wszystkie osobniki zmieniają sezonowo swój fenotyp. W jednej populacji możemy wyróżnić osobniki odpowiadające, które wykształcają wszystkie cechy zimowego fenotypu (zmniejszona masa ciała, regresja gonad i zahamowanie rozmnażania, zmiana okrywy włosowej czy wykorzystywanie odrętwień), osobniki nieodpowiadające utrzymujące przez cały rok cechy letniego fenotypu oraz całe spektrum osobników o pośredniej odpowiedzi, które mogą wykazywać tylko niektóre z cech zimowego fenotypu. Celem tej pracy była weryfikacja hipotez tłumaczących powstawanie i utrzymywanie się polimorfizmu fenotypu zimowego w populacji zwierząt heterotermicznych. Wykorzystałam chomicznika dżungarskiego (*Phodopus sungorus*), który jest modelowym gatunkiem w badaniach nad sezonowymi zmianami fenotypu. Przetestowałam korelacje zimowego fenotypu z międzyosobniczymi różnicami w behawiorze, metabolizmie energetycznym i cechach historii życiowych zwierząt. Wykazałam, że osobniki o różnych zimowych fenotypach nie różnią się behawiorem i podstawowym tempem metabolizmu, ale osobniki odpowiadające charakteryzują się mniejszą elastycznością fenotypową tych cech (Artykuł I). Z kolei osobniki nieodpowiadające wcześniej przystępują do rozrodu i wydają na świat większe młode (Artykuł II). Ponadto, wykazałam, że wykształcenie zimowego fenotypu może być związane z czasem urodzenia w ciągu sezonu rozrodczego oraz po raz pierwszy u chomiczników dżungarskich opisałam polimorfizm zimowego fenotypu wewnątrz jednego miotu (Artykuł III). Na podstawie uzyskanych wyników wnioskuję, że każdy z fenotypów jest korzystny i ma przewagę nad pozostałymi w odmiennych warunkach środowiska. Nieprzewidywalne zmiany warunków środowiska, w tym te wynikające ze zmian klimatycznych, mogą więc faworyzować, a w konsekwencji utrwalać polimorfizm zimowego fenotypu wewnątrz populacji.

2. Summary

Polymorphism is the occurrence of different phenotypes within a population of a species in which individuals can mate randomly. In seasonal environment animals change their phenotype reversibly to adjust their physiology and behaviour to environmental conditions. Animals living in Temperate and Boreal Zones develop winter phenotype in response to shortening days. Since development of winter phenotype lead to lower energy expenditure, it should be favoured by natural selection. Nevertheless not all individuals change their phenotype in winter. Within one population we can observe responding individuals, which develop all winter traits (reduced body mass and gonadal regression, changed colour and properties of fur, heterothermy), nonresponding individuals maintaining summer phenotype throughout the year, and whole spectrum of individuals presenting only some of winter traits. The aim of this thesis was to verify hypotheses explaining development and maintenance of winter phenotype polymorphism in heterothermic species. I used Siberian hamster (*Phodopus sungorus*), a model species to study both seasonality and polymorphism of winter phenotype. I tested correlation between development of winter phenotype and individual differences in behaviour, metabolism and life histories. I found that phenotypes did not differ in behaviour or basal metabolism, but responding individuals had lower phenotypic flexibility of these traits (Article I). Nonresponding individuals commenced breeding earlier and produced bigger offspring (Article II). Moreover, I found that development of winter phenotype may be related to time of birth during reproducing season. Finally, I described, for the first time polymorphism of winter phenotype within a litter of Siberian hamster (Article III). Based on these results I suggest that each phenotype is beneficial and may be favoured under different environmental conditions. Unpredictable changes of environmental conditions, including those resulting from climate change, may favor and therefore maintain polymorphism of winter phenotype in the population.

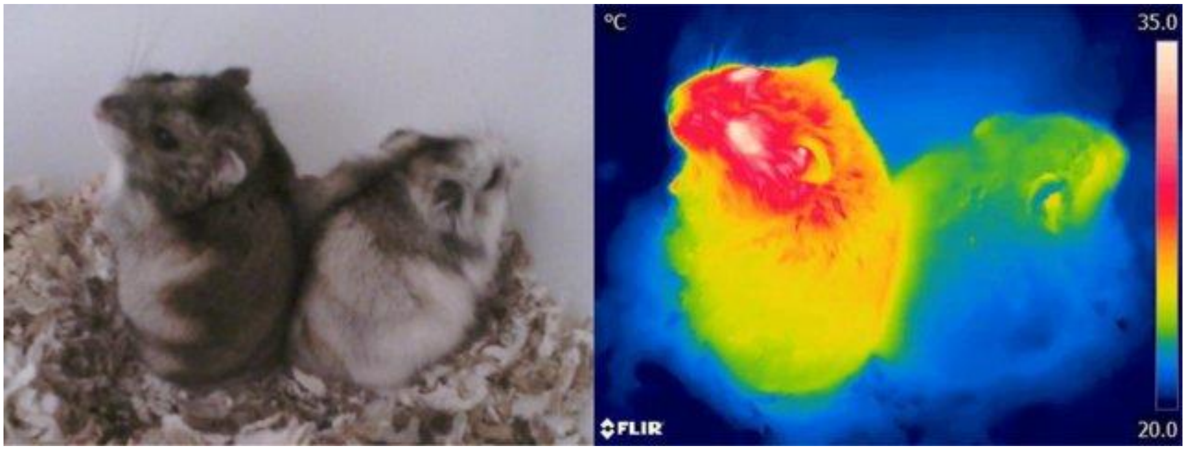
3. Komentarz autora

Zima charakteryzująca się krótkim dniem, niską temperaturą otoczenia i ograniczonym dostępem do pokarmu jest najtrudniejszym okresem w ciągu roku, szczególnie dla małych gatunków endotermicznych, u których wysokie tempo metabolizmu wymaga dużych nakładów energetycznych (Ruf et al., 2012a; Schmidt-Nielsen, 2008). W odpowiedzi na skracający się fotoperiod (jasna faza doby), który jest sygnałem informującym o nadchodzącej zimie (Bartness i Goldman, 1989; Wood i Loudon, 2014), małe ssaki zasiedlające strefę klimatów umiarkowanych zmieniają swój fenotyp na zimowy (Arnold i Rubin, 2009; Heideman et al., 1999; Hoffmann, 1978; Hoffmann i Illnerova, 1986; Majoy i Heideman, 2000; Mills et al., 2018; Moffatt et al., 1993; Ruf et al., 2012a; Williams et al., 2017; Zimova et al., 2018). Wykształcenie zimowego fenotypu jest przykładem elastyczności fenotypowej, czyli zdolności do odwracalnej zmiany fenotypu (Piersma i Drent, 2003; Piersma i Van Gils, 2011). Pozwala to obniżyć wydatki energetyczne w odpowiedzi na zmieniające się, niekorzystne warunki środowiska. Do cech zimowego fenotypu małych ssaków należą: obniżona masa ciała (Heideman i Bronson, 1991; Li i Wang, 2005; Steinlechner et al., 1983), zahamowanie rozmnażania przez regresję gonad lub inwolucję całego układu rozrodczego (Bronson i Perrigo, 1987; Butler et al., 2010; Nelson, 1985a), czy zmiana okrywy włosowej i zwiększenie jej właściwości izolacyjnych (Duncan i Goldman, 1984b; Heath i Lynch, 1982; Severaid, 1945). Ponadto gatunki przemiennocieplne (heterotermiczne) mogą wykorzystywać odrętwienia jako dodatkowy sposób redukcji wydatków energetycznych (Heldmaier i Steinlechner, 1981; Jastroch et al., 2016; Levesque i Tattersall, 2010). Mimo, że zimowy fenotyp powinien być faworyzowany przez dobór naturalny, nie wszystkie osobniki w populacji odpowiadają na skracający się fotoperiod zmianą fenotypu (Ford, 1945; Mills et al., 2018; Nelson, 1987).

Zjawisko polimorfizmu wewnątrz jednej populacji występuje wówczas, kiedy osobniki zasiedlające to samo środowisko w tym samym czasie i mogące się swobodnie krzyżować prezentują różne fenotypy (Ford, 1945; Huxley, 1955; Leimar, 2005). Jednym z przykładów polimorfizmu występującego w warunkach naturalnych jest polimorfizm zimowego fenotypu (Nelson, 1987; Prendergast et al., 2001). Jest on obserwowany u wielu gatunków ssaków, w tym u przedstawicieli zajęczaków (czterosiekaczowców), gryzoni i drapieżnych (Ford, 1945; Mills et al., 2018; Nelson, 1987; Prendergast et al., 2001; Zimova et al., 2018). Populacje tych gatunków składają się z osobników odpowiadających na skracający się fotoperiod wykształceniem cech charakterystycznych dla zimowego fenotypu oraz osobników, które nie odpowiadają na skracający się fotoperiod, utrzymując przez cały rok cechy letniego fenotypu czyli większą masę ciała, aktywne gonady i wysoką temperaturę ciała. Ponadto istnieje cała gama osobników o fenotypie pośrednim, różniących się występowaniem poszczególnych cech, ich nasileniem czy czasem ich wykształcenia (Mills et al., 2018; Nelson, 1987; Prendergast et al., 2001; Zimova et al., 2014).

Mimo wielu badań do tej pory jednoznacznie nie ustalono co leży u podstaw wykształcenia różnych zimowych fenotypów w populacji. Wiadomo, że występowanie polimorfizmu zimowego fenotypu nie jest związane z płcią (Puchalski i Lynch, 1991), ale zimowy fenotyp jest odziedziczalny (Diedrich et al., 2015; Goldman et al., 2000; Heideman et al., 1999; Lynch et al., 1989). Brak odpowiedzi na krótki fotoperiod może być też indukowany, np. ekspozycją na fotoperiod dłuższy niż 18h (Goldman i Goldman, 2003; Gorman i Zucker, 1997). Wykazano również, że zdolność do odpowiedzi na krótki fotoperiod maleje z wiekiem (Bernard et al., 1997; Freeman i Goldman, 1997; Horton i Yellon, 2001), zaś intensywność odpowiedzi wzrasta w niekorzystnych warunkach środowiska, tj. w niskiej temperaturze otoczenia, lub w czasie braku dostępu do pokarmu (Lynch, 1973; Prendergast et al., 2001; Ruf et al., 1993). Przyczyn braku odpowiedzi na krótki fotoperiod upatrywano również w funkcjonowaniu zegara biologicznego i szyszynki. Nieodpowiadające myszaki białostope (*Peromyscus leucopus*) nie różnią się poziomem produkowanej melatoniny od myszaków odpowiadających, lecz receptory melatoniny u osobników nieodpowiadających są na nią niewrażliwe (Blank et al., 1992; Blank et al., 1988). Z kolei nieodpowiadające chomiczniki dżungarskie mają nocą niższy poziom melatoniny niż osobniki odpowiadające (Freeman i Goldman, 1997; Puchalski i Lynch, 1986; Puchalski i Lynch, 1988). W odpowiedzi na egzogenną melatoninę chomiczniki odpowiadające zaaklimowane do długiego fotoperiodu wykształcają cechy charakterystyczne dla zimowego fenotypu (Bartness i Goldman, 1988; Boratyński et al., 2017b). Podanie melatoniny chomicznikom nieodpowiadającym na krótki fotoperiod prowadzi natomiast do wykształcenia zimowego fenotypu tylko u niektórych osobników, podczas gdy u innych nie powoduje takich zmian. Wskazuje to, że przynajmniej część osobników nieodpowiadających jest niewrażliwa na melatoninę (Boratyński et al., 2017b; Puchalski i Lynch, 1986; Puchalski i Lynch, 1988). Ponadto część chomiczników nieodpowiadających różni się od osobników odpowiadających rytmiką okołodobową (Puchalski i Lynch, 1986; Puchalski i Lynch, 1988) oraz wzorcem aktywności bioelektrycznej neuronów jąder nadskrzyżowaniowych podwzgórza (Margraf et al., 1991). Nie wiadomo również dlaczego część chomiczników po aklimacji do krótkiego fotoperiodu wykazuje tylko niektóre z cech zimowego fenotypu (Butler et al., 2007; Jefimow et al., 2020). Powyższe badania nie dają więc jednoznacznej odpowiedzi na pytania o to jak powstają i czym różnią się zimowe fenotypy chomiczników. Choć wiadomo, że to krótki fotoperiod jest czynnikiem inicjującym zmianę fenotypu, to pomimo wielu badań do tej pory nie ustalono bezpośrednich i ultymatywnych przyczyn występowania i utrzymywania się polimorfizmu zimowego fenotypu w obrębie populacji.

Celem cyklu prac przedstawionych w niniejszej rozprawie doktorskiej jest weryfikacja hipotez wyjaśniających występowanie i utrzymywanie się zjawiska polimorfizmu zimowego fenotypu w populacji heterotermicznego gryzonia, chomicznika dżungarskiego (*Phodopus sungorus*). Chomiczniki są gatunkiem modelowym w badaniach nad sezonowymi zmianami fenotypu (Bao et al., 2019; Butler et al., 2010; Gorman i Zucker, 1995; Petri et al., 2016; Steinlechner i Heldmaier, 1982). Podczas aklimacji lub aklimatyzacji do warunków zimowych (krótki fotoperiod albo krótki fotoperiod i niska temperatura otoczenia) osobniki odpowiadające wykazują wiele cech zmniejszających wydatki energetyczne organizmu, tj. redukcję masy ciała, regresję gonad, zmianę koloru i jakości futra oraz odrętwienia dobowe (Figala et al., 1973; Hoffmann, 1978; Hoffmann i Illnerova, 1986). Ponadto w obrębie populacji chomiczników istnieją również osobniki nieodpowiadające (Ryc. 1) oraz wykazujące tylko niektóre cechy zimowego fenotypu (Butler et al., 2007; Gorman i Zucker, 1997; Lynch et al., 1989; Puchalski i Lynch, 1986). Proponuję, że polimorfizm zimowego fenotypu jest utrzymywany w populacji, ponieważ wszystkie fenotypy mogą być korzystne w zależności od warunków środowiska.



Rycina 1. Samice chomicznika dżungarskiego, pochodzące z tego samego miotu i utrzymywane w tych samych warunkach, prezentujące różne zimowe fenotypy po 14 tygodniach aklimacji do krótkiego fotoperiodu. Samica po lewej stronie nie odpowiadała na krótki fotoperiod (szare futro, normotermia), podczas gdy samica po prawej stronie zmieniła fenotyp na zimowy (białe futro, torpor; Jefimow et al., 2020).

W pierwszej pracy (Artykuł I) testowałam hipotezę mówiącą, że polimorfizm zimowego fenotypu jest związany z międzyosobniczymi różnicami w behawiorze i tempie metabolizmu zwierząt. Ponieważ fizjologia i zachowanie zwierząt mogą być ze sobą związane (Careau et al., 2015; Careau et al., 2008; Reale et al., 2010) zaproponowałam również, że różnice w fizjologii i behawiorze zwierząt prezentujących odmienne fenotypy są ze sobą skorelowane. Przewidywałam, że osobniki nieodpowiadające na skracający się fotoperiod, niezmieniające zimą fenotypu i niewykorzystujące odrętwień dobowych prezentują osobowość proaktywną, czyli są bardziej aktywne i odważne. Powinny więc mieć też wyższe wydatki energetyczne, a tym samym i tempo metabolizmu niezależnie od sezonu. Z kolei osobniki wykształcające cechy zimowego fenotypu powinny charakteryzować się osobowością reaktywną, strachliwą, co wraz ze zdolnością do wykorzystywania dobowych odrętwień w czasie zimy prowadzi do niższych wymagań energetycznych i niższego tempa metabolizmu niż w przypadku zwierząt nieodpowiadających.

W drugiej pracy (Artykuł II) testowałam związek polimorfizmu zimowego fenotypu z cechami historii życiowych zwierząt. Miarą sukcesu ewolucyjnego jest dostosowanie, czyli wypadkowa długości życia i sukcesu reprodukcyjnego osobnika (Łomnicki, 2009). Pomimo, że wykształcenie cech zimowego fenotypu prowadzące do oszczędzania energii w niesprzyjających warunkach środowiska powinno zwiększać dostosowanie zwierząt i być faworyzowane przez dobór naturalny, to nie ma bezpośrednich dowodów na większą przeżywalność osobników odpowiadających na krótki fotoperiod. Istnieją natomiast badania łączące wykorzystywanie odrętwień z wydłużeniem życia czy spowolnieniem tempa starzenia (Place i Cruickshank, 2009; Place et al., 2004; Ruf et al., 2012b; Turbill et al., 2012). Jeśli osobniki nieodpowiadające, niewykorzystujące odrętwień dobowych, charakteryzują się krótszym życiem, a co za tym idzie niższym dostosowaniem, to dobór naturalny powinien je eliminować. Jednak są one obecne w populacji, co wskazuje na podobne dostosowanie obu fenotypów. W takim przypadku sukces reprodukcyjny osobników nieodpowiadających powinien być większy niż osobników odpowiadających. Postawiłam więc hipotezę, że zimowe fenotypy różnią się cechami historii życiowych. Przewidywałam, że osobniki nieodpowiadające, które nie redukują gonad w czasie zimy, przystępują do rozrodu wcześniej i wydają na świat większe mioty z mniejszymi młodymi niż osobniki odpowiadające. Ponadto jeśli osobniki nieodpowiadające i odpowiadające na krótki fotoperiod różnią się cechami historii życiowych, sugerowałam, że różnice te mogą wpłynąć na inwestycje w utrzymanie somatyczne, co może skutkować różnicami w statusie oksydacyjnym po reprodukcji.

W trzeciej pracy wchodzącej w skład rozprawy doktorskiej (Artykuł III) testowałam związek pomiędzy cechami historii życiowych a wykształceniem zimowego fenotypu. Zgodnie z teorią opóźnionego oddziaływania cech historii życiowych na fenotyp dorosłego osobnika, oprócz genów, wpływ mają warunki środowiska jakich zwierzę doświadczyło w początkowym okresie życia (Beckerman et al., 2002; Prevot-Julliard et al., 1999; Searcy et al., 2014). Jednym z takich czynników jest czas urodzenia w ciągu sezonu rozrodczego (Butler et al., 2008; Rogovin et al., 2014; Searcy et al., 2014). Zwierzęta urodzone wiosną różnią się od tych urodzonych latem lub jesienią masą ciała, tempem wzrostu i dojrzewania, przeżywalnością i sukcesem reprodukcyjnym, czy strategią przetrwania zimy (Albon et al., 1987; Clutton-Brock et al., 1987; Ergon et al., 2001; Helle et al., 2012; Huck et al., 1986; Searcy et al., 2014). W swojej pracy przetestowałam eksperymentalnie efekt późnego urodzenia w ciągu roku na wykształcenie zimowego fenotypu. Zaproponowałam, że dostęp do zasobów energetycznych przed rozpoczęciem zimy może wpływać na wykształcenie odpowiedzi na krótki fotoperiod i postawiłam hipotezę, że osobniki odpowiadające będą występowały częściej wśród zwierząt urodzonych później w ciągu sezonu rozrodczego, niż wśród tych urodzonych wcześniej. Osobno przetestowałam wpływ kolejności miotu, w którym zwierzęta przyszły na świat oraz wpływ długości aklimacji do długiego fotoperiodu. Przewidywałam, że proporcja zwierząt prezentujących cechy zimowego fenotypu będzie większa wśród osobników, które spędziły mniej czasu w długim fotoperiodzie, niezależnie od miotu z którego pochodzą.

4. Artykuły będące podstawą rozprawy doktorskiej:

Przybylska A. S., Wojciechowski M. S. , Jefimow M. (2019) Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behavior, Animal Behaviour 147:105-114; doi:10.1016/j.anbehav.2018.11.007 (**Artykuł I**).

Przybylska A. S. , Wojciechowski M. S. , Jefimow M. (2019) Photoresponsiveness affects life history traits but not oxidative status in a seasonal rodent, Frontiers in Zoology 16:11; doi:10.1186/s12983-019-0311-3 (**Artykuł II**).

Przybylska-Piech A. S. , Wojciechowski M. S. , Jefimow M. (2021) Consecutive litters do not differ in photoresponsiveness but prolonged acclimation to long photoperiod inhibits winter molt, Frontiers in Zoology18:11; doi: 10.1186/s12983-021-00391-3 (**Artykuł III**).

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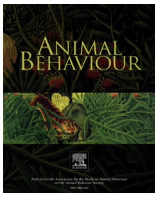

Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behaviour

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photoresponsiveness
Siberian hamster

Living in a seasonally changing environment requires periodic, reversible changes in animals' phenotypes to match variations in their abiotic and biotic environments. These changes may relate to temperature regulation, torpor use, basal metabolic rate, body mass or behaviour, all acting in concert to ensure the best adjustment to the environmental challenges. As winter approaches, many small mammals develop a winter phenotype that is qualitatively and quantitatively different from a summer one. However, there is a significant within-population polymorphism in winter phenotype. We hypothesized that winter phenotype is correlated with consistent between-individual differences in animal behaviour, i.e. animal personality, and energy metabolism. We measured basal metabolic rate (BMR) and behavioural traits in three winter phenotypes of Siberian hamsters, *Phodopus sungorus*, which were acclimated to summer-like and then to winter-like conditions: fully responding, nonresponding and partially responding to a short photoperiod. We found no differences in behavioural traits between hamsters of different winter phenotypes, but the seasonal increase in activity was lowest in full responders indicating their lower behavioural flexibility than partial responders and nonresponders. The same was true for BMR. While nonresponders and partial responders increased their BMR from summer to winter, full responders did not change it. We argue that different winter phenotypes are maintained in the population because they could be beneficial under different environmental conditions. We also suggest that within a population there is a continuum of winter phenotypes, which is not related to differences in animal personalities. This continuum allows for population maintenance despite environmental conditions changing over short and long timescales.

In response to shortening days and falling ambient temperature (T_a) many small temperate-zone animals change to an energy-conserving winter phenotype. They show several changes that lead to significant energy conservation. They regress gonads (Broussard et al., 2008; Kaseloo, Crowell, & Heideman, 2014), decrease body mass, m_b (Hoffman, 1973; Moffatt, DeVries & Nelson, 1993; Bernard, Losee-Olson, & Turek, 1997), change their fur's colour and properties (Heldmaier & Steinlechner, 1981a; Freeman & Goldman, 1997b; Goldman, Dhandapani, & Goldman, 2000) and may enter daily torpor (Hoffman, 1973; Heldmaier & Lynch, 1986; Lynch & Puchalski, 1986; Geiser & Ruf, 1995). The development of this adaptive syndrome (Heldmaier & Lynch, 1986) is driven mainly by changes in photoperiod. However, despite apparent advantages of the winter phenotype, not all individuals respond to a short photoperiod in the same way (Nelson, 1987; Prendergast, Kriegsfeld, & Nelson, 2001). Within populations of photosensitive species there is a considerable polymorphism of the winter phenotype: some individuals respond fully to shortening days (full responders) and others are insensitive to changes in daylength (nonresponders; Nelson, 1987; Prendergast et al., 2001). Between these two extremes, a spectrum of intermediate phenotypes can be found. We classify these animals as partial responders (Przybylska, Wojciechowski, Drobniak, & Jefimow, 2017). The Siberian hamster, *Phodopus sungorus*, which is a highly seasonal rodent, is a perfect example of winter phenotype polymorphism. Full responders enter daily torpor and have a reduced m_b, regressed gonads and white fur while nonresponders do not show these changes (Figala, Hoffmann & Goldau, 1973; Hoffmann, 1973; Heldmaier & Steinlechner, 1981a,b; Lynch & Puchalski, 1986; Ruf & Heldmaier, 1992; Ruf, Stieglitz, Steinlechner, Blank, & Heldmaier, 1993); partial responders change only partially, for example, they may reduce only

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m_b or they may change m_b and fur coloration, they may change m_b and enter daily torpor or they may change fur coloration but not enter torpor (Puchalski & Lynch, 1986; Kliman & Lynch, 1992; Larkin, Freeman, & Zucker, 2001; Przybylska et al., 2017, S. Steinlechner, personal communication, February 2016). Polymorphism of the winter phenotype is a phenomenon pertinent not only to Siberian hamsters (Heldmaier & Steinlechner, 1981b; Hoffmann, 1973; Ruf et al., 1993) but also to other mammal species. Among rodents it has been described in pouched mice, *Saccostomus campestris* (Ellison, 1993), pocket mice, *Perognathus californicus* (Tucker, 1965), white-footed mice, *Peromyscus leucopus* (Broussard et al., 2009; Heath & Lynch, 1983; Kaseloo et al., 2014; Lynch, 1973), deer mice, *Peromyscus maniculatus* (Moffat et al., 1993), meadow voles, *Microtus pennsylvanicus*, and prairie voles, *Microtus ochrogaster* (Karebeshian et al., 1994; Nelson, 1987).

Several explanations for the existence of the polymorphism in winter phenotype have been suggested. Different photoresponsiveness may be a result of differences in melatonin synthesis (Puchalski & Lynch, 1986), different photoperiodic histories (Gorman & Zucker, 1997; Goldman & Goldman, 2003) or differences in circadian organization (Puchalski & Lynch, 1986; Freeman & Goldman, 1997a). It is known that winter phenotype is heritable (Lynch, Lynch, & Kliman, 1989, Goldman et al., 2000; Goldman & Goldman, 2003; Diedrich, Bank, Scherbarth, & Steinlechner, 2015), and depends on age (Bernard et al., 1997; Freeman & Goldman, 1997a) and environmental conditions (Heldmaier & Steinlechner, 1981a, b; Anchordoquy & Lynch, 2000). Finally, Ruf and Heldmaier (2000) suggested that photoresponsiveness may be related to behaviour, and that nonresponding Siberian hamsters are more active and more prone to take risky behaviours than fully responding ones.

Here we tested the hypothesis that physiological differences between winter phenotypes, fully responding, partially responding or nonresponding to a short photoperiod, correlate with differences in behaviour. Our reasoning was based on the fact that animals of different personalities differ in behavioural, neuroendocrine and physiological characteristics (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Proactive animals are more aggressive, active and bold than reactive individuals which are shy, less active and less aggressive. On the slow–fast continuum proactive animals would be considered 'fast', whereas reactive individuals would be 'slow' (Benus, Bohus, Koolhaas, & van Oortmerssen, 1991; Careau, Thomas, Humphries, & Réale, 2008; Koolhaas et al., 1999; Réale et al., 2010, 2007). Because personality results from consistent between-individual differences in behaviour we expected that these between-phenotype differences would be consistent throughout a year. We predicted that nonresponders, which do not change their phenotype for winter and do not enter torpor, represent a bold, proactive personality, while full responders, which enter torpor, demonstrate a shy, reactive personality. At the same time, since behaviour may change seasonally (Rusak & Zucker, 1975), we expected to observe summer-to-winter changes in behavioural traits, but not in phenotype personality. As heterothermy, defined as a significant deviation in body temperature (T_b) from its modal value, is relatively common among mammalian taxa, especially among small species in winter (Boyles et al., 2013), and as animals differ in personality (Gosling, 2001), one might expect a link between behavioural traits and heterothermy, that is, torpor use. Furthermore, several authors have postulated a relationship between personality and energetics (Careau et al., 2015, 2008; Careau & Garland, 2012). Because full responders change to an energy-conserving winter phenotype we predicted that they would have smaller energy requirements resulting in lower basal metabolic rate (BMR) than nonresponders.

The polymorphism of winter phenotype among Siberian hamsters makes this species a perfect model for testing these hypotheses. To verify our predictions, we measured BMR and behavioural traits in Siberian hamsters acclimated to summer-like and then to winter-like conditions, and which developed different winter phenotypes, to outline the possible explanations for a correlation between energy metabolism, heterothermy use and behaviour.

METHODS

Ethical Note

All experiments received ethical approval from the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decision numbers 3/2015, 31-33/2015, 35/2015).

Animals and Housing

Siberian hamsters used in these experiments originated from our breeding colony which consists of animals descended from hamsters obtained from the University of Halle-Wittenberg and Philipps University of Marburg, Germany. We used 160 adult hamsters (80 males and 80 females) born under summer-like conditions (16:8 h light:dark and $T_a = 20 \pm 2$ °C). The summer series of experiments was done when animals were ca. 3 months old. After that, when hamsters were ca. 5 months old, they were transferred to winter-like conditions ($T_a = 10$ °C, 8:16 h light:dark, lights on at 0830) and the winter series of experiments was done after 15–19 weeks of acclimation. Under both summer and winter acclimation regimes hamsters were housed individually in standard laboratory cages (33 × 20 cm and 18 cm high) with wood shavings as bedding; food (standard rodent diet; Labofeed B, Morawski, Kcynia, Poland) and water were available ad libitum. In winter, diet was supplemented with ca. 4 g of sunflower seeds every week. We did not provide cage enrichment because this could affect the results of behavioural and metabolic measurements. Every 2 weeks hamsters were weighed to ±0.1 g with an electronic balance (SPU402, Ohaus, U.S.A.) to monitor changes in m_b during acclimation. We also measured m_b before and after each metabolic and behavioural measurement.

Defining Hamster Phenotype

The summer series of experiments was done on hamsters of unknown photoresponsiveness status, which could be determined only after acclimation to winter-like condition. Hamsters were defined as full responders, nonresponders or partial responders from T_b recordings and fur coloration. As a result of acclimation to winter-like conditions, full responders entered daily torpor and moulted to a white fur, nonresponders did not enter daily torpor and remained grey throughout winter, and partial responders changed to a white fur, but did not enter daily torpor or remained grey but entered a few torpor episodes. This classification resulted in a highly diverse group of partial responders which showed different combinations of seasonal changes in morphology and physiology. We consider, however, that this was the best solution and allowed for data analysis without losing information on the individuals that could not be unambiguously classified as full responders or nonresponders.

Body temperature was recorded every 15 min with miniature data loggers (model TL3-1-27, mass 0.8 g, accuracy of 0.3 °C from 0 °C to 45°C, D. Petrovsky, Russian Academy of Sciences, Novosibirsk, Russia). The data loggers were implanted subcutaneously under ketamine (40 mg/kg; Ketamina 10%, Biowet, Puławy, Poland) and xylazine (8 mg/kg; Sedazin 2%, Biowet, Puławy, Poland) anaesthesia before acclimation to winter-like conditions. Before implantation, all loggers were covered with paraffin wax (final mass ca. 0.9 g, between 2.8 and 3.6% of hamster m_b) and calibrated against a precise mercury-in-glass thermometer to the nearest 0.3 °C. After completing all experiments, 60 hamsters were euthanized by cervical dislocation, and the data loggers were removed. In the remaining 100 hamsters, which were used for breeding, data loggers were removed under anaesthesia as described above.

Respirometry

In summer and winter, the BMR of normothermic hamsters was measured by indirect calorimetry using an open flow respirometry system, at $T_a = 28.5$ °C, which is within the thermoneutral zone of Siberian hamsters (Gutowski, Wojciechowski, & Jefimow, 2011). Measurements commenced at approximately 0900 hours, that is, during the animals' rest phase, and lasted for ca. 7 h. We used two parallel respirometry systems that allowed us to measure gas exchange in 14 hamsters simultaneously. The system was set up so that the respiratory gas exchange of two animals was measured in parallel. Recordings were done for 5 min with reference air being sampled for 4 min at least every 15 min. As a result, the respiratory gas exchange was measured in each hamster every 44 min throughout the experiment. Outside air was pulled from outside the building using an air pump and compressed in a balloon, then dried and scrubbed of CO_2 with a PureGas Generator (Puregas, Westminster, CO, U.S.A.). Next, air was continuously pushed through 0.85-litre airtight metabolic chambers constructed of translucent polypropylene containers (HPL 808, Lock & Lock, Hana Cobi, South Korea), with a constant flow rate of ca. 330 ml/min. All chambers were placed in a temperature-controlled cabinet (ST-1200, Pol-Eko-Aparatura, Wodzisław Śląski, Poland). Airflow was regulated upstream of the respirometry chambers using precise needle valves. Gases leaving the respirometry chambers were selected sequentially with a computer-controlled multiplexer (MUX, Sable Systems Int., Las Vegas, NV, U.S.A.) and flow rate though a selected chamber was measured downstream using a mass flow meter (FlowBar-8, Sable Systems Int.). A multiplexer was set up so that two parallel, independent air streams leading to two gas analysers were selected at the same time. After flow measurement, air from each gas stream was subsampled at a rate of ca. 100 ml/min and water vapour pressure of the subsampled air was measured with a water vapour analyser (RH-300, Sable Systems Int.). Air was then dried in a column of magnesium perchlorate (Sigma-Aldrich, U.S.A.), and subsequently fractional concentrations of CO_2 (FCO_2) and O_2 (FO_2) were measured using a FoxBox-C integrated CO_2 and O_2 analyser, or with a FC-10a O_2 analyser (Sable Systems Int.) and CA10 CO_2 analyser (Sable Systems Int.). All electronic elements of the respirometry system were connected to a PC computer via an analogue-to-digital interface (UI2, Sable Systems Int.). Respirometry data were acquired using ExpeData software (Sable Systems Int.) at 0.5 Hz and were calculated using equations 11.7 and 11.8 in Lighton (2008).

Metabolic rate (MR, in watts) was calculated assuming $\mathrm{RER} = (\dot{V}\,CO_2/\dot{V}\,O_2)$ calculated from recorded $\dot{V}\,CO_2$ and $\dot{V}\,O_2$ using the oxyjoule equivalent after Lighton, Bartholomew, and Feener (1987) as follows:

$$\mathrm{MR(W)} = \frac{\dot{V}\mathrm{O_2}(16 + 5.164 \cdot \mathrm{RER})}{60}$$

where $\dot{V}\,O_2$ is the rate of oxygen consumption (ml O_2/min).

Open Field

To test behavioural traits, we used the open field (OF) test which allowed us to quantify activity, exploration and stress response in a novel environment (Réale et al., 2007; Walsh & Cummins, 1976). In both seasons (summer and winter) each hamster was subjected to the test twice, 10 days apart (Réale et al., 2007). Animals were tested during the light phase between 0900 and 1500 hours in an arena (80×80 cm and 40 cm high) made from white opaque Plexiglas, placed under a white cotton fabric tent which diffused light equally. All sources of light were set outside the tent. The arena was illuminated by a 150 W bulb placed at a height of 2 m and four 150 W bulbs located outside the corners of the apparatus, resulting in 100–120 lx inside the arena. Outside the arena we placed two fans which generated white noise during the experiments. After each trial, the arena was cleaned with 70% ethanol. A hamster was placed in a corner of the arena in an opaque transport box with sliding doors. After 1 min the box was opened, and the hamster could enter the OF voluntarily. If it did not do so within 5 min, it was removed from the analyses. The transport box was removed after the hamster left it. Test sessions were recorded with a digital camera (GigE monochrome Basler acA1300-60gmNIR, Basler, Ahrensburg, Germany), placed next to the lamp mounted above the experimental set-up, for 5 min after the hamster left the transport box. The analysis of these recordings was done with EthoVision version 11.5 (Noldus Information Technology, Wageningen, Netherlands). The total distance covered in the arena and total time spent near the walls were analysed automatically, while the total duration of scratching at the arena, rearing, grooming and climbing the walls were scored manually by A.S.P. Repeatability of manual scoring ranged between 0.85 and 0.95.

Data Analysis

We collected a full set of data from 152 animals of which 34 were full responders, 96 were nonresponders and 22 were partial responders.

To test whether phenotypes differed in behaviour and whether this correlated with metabolism, we used a Markov chain Monte Carlo multivariate mixed model in R v. 3.4.1 using MCMCglmm package v. 2.24 (CRAN, Hadfield, 2010). We used a Bayesian approach because it allowed us to estimate within- and between-individual variance and covariance of traits, as well as the 95% credible interval, which does not require randomization or bootstrapping procedures (Nakagawa & Schielzeth, 2010). First, we ran univariate models to calculate repeatability of behavioural traits. To do so, we decomposed the variance into within-individual (V_W) and among-individual (V_A) components, and repeatability (r) was calculated as $\frac{V_A}{V_A+V_W}$. We assessed repeatability of six behavioural traits: total distance covered in the arena, total time spent near the walls and total duration of scratching at the arena, rearing, grooming and climbing the walls. Following Roche, Careau, and Binning (2016), we calculated agreement repeatability, which is the proportion of phenotypic variance due to differences between individuals (model with individual ID as a random factor and without fixed factors). Then we added fixed factors to the model and obtained adjusted repeatability. We used phenotype, season, trial number and sex as fixed factors. We also added second-order interactions to the model (phenotype*season, phenotype*sex, trial*season, sex*season) to control for their potential influence on the variability of analysed traits. To asses whether repeatability differed between phenotypes we used idh variance structure in random effects which assumes independence of groups, fixes covariance to 0 and allows for different random effect variation depending on the phenotype (Hadfield, 2017). Because we did not have previous knowledge about parameter distributions in univariate models, we used weakly informative priors. Thus, all the information in the analysis comes from the data. We ran univariate models with various priors but finally we used a weakly informative prior for variance ($V = 1$, $\eta = 2$) and an uninformative prior for the correlations. We ran models for 325 000 iterations, after an initial burn-in with 75 000 iterations and a 250 thinning interval (Hadfield, 2017).

To test whether phenotypes differed in their behaviour we used univariate models for behavioural scores obtained in principal components analysis (PCA). This was done using the nFactors package in R (v. 2.3.3, Raiche, 2010). First, we used the Kaiser rule (Kaiser, 1991) to determine the number of components to retain. We obtained three PCs that in total explained 74% of the initial variance of the data. These were identified as proactive behaviour, anxiety and high stress reaction (see Results). We defined initial mixed-effects models with the same set of fixed factors and interactions as in single behavioural trait models which were then reduced for nonsignificant factors. We considered fixed factors to be statistically significant if their 95% highest posterior densities (HPD) intervals did not include zero. For the model selection we used the deviance information criterion (DIC) which informs about the goodness-of-fit and complexity of the model, with the lowest DIC indicating the better model (Hadfield, 2017). Repeatability of proactive behaviour, anxiety and high stress response was calculated for the models with the lowest DIC.

For negative results, to infer the accuracy of parameter estimates (Kelley, Maxwell, & Rausch, 2003) we used the pMCMC value, which is the doubled probability that a test statistic in the reference distribution exceeds its value in the obtained data.

To test for the effect of phenotype on m_b and BMR in winter and summer, we ran a general linear model (GLM) in IBM SPSS Statistics v. 24 (IBM Corp., Armonk, NY, U.S.A.). The model for m_b included phenotype, season and sex as fixed factors and the phenotype*season interaction. The model for BMR included the same fixed factors, m_b as covariate, and phenotype*season and phenotype*sex interactions. These models allowed us to estimate the significance of fixed factors on m_b and m_b-adjusted BMR. These data are presented as estimated marginal means ± SE.

Finally, we ran bivariate mixed models to estimate the correlation between physiology and behaviour. Because we used behavioural traits measured in one test, we decided to fit three separate bivariate models instead of one multivariate model. In each model we used BMR and one PC score for behaviour as dependent variables. In the initial model we included phenotype, sex and season as fixed factors and interactions of phenotype and season, phenotype and sex, and sex and season. Additionally, m_b was included as the covariate for BMR and trial as a fixed factor to the model analysing behaviour to control for the effect of consecutive measurements (see Results). Models were reduced by removing nonsignificant factors and were compared using DIC. Then we chose the best fitting model for further analysis. We calculated among (r_{ind}) and within (r_e) individual correlations between BMR and behavioural traits, as $\frac{COV}{\sqrt{V_{x1} \times V_{x2}}}$, where COV is the covariance of two traits and V_{x1} and V_{x2} are variances of analysed traits. The among-individual correlation informs about the relationship between two traits that are stable in the population over time. The within-individual correlation represents any changes within individuals, which are related to short-term relationships between two traits (Dingemanse & Dochtermann, 2013). To test whether a correlation between physiology and behaviour was phenotype dependent, we fitted separate variance–covariance matrices for each phenotype. These analyses were done in R v. 3.4.1.

RESULTS

Repeatability of Behaviour

Agreement repeatability showed that behavioural traits were repeatable (e.g. grooming: HPD 0.12–0.27; total distance covered in the arena: HPD 0.28–0.45; Table 1). Adjusted repeatability was higher than 0.20 for all traits and for total distance covered in the arena reached 0.43 (HPD 0.34–0.51). In models with variation separated by phenotype, fully responding hamsters showed the highest repeatability in climbing (HPD 0.38–0.67), grooming (HPD 0.23–0.53) and rearing (HPD 0.14–0.43). Nonresponding hamsters showed the highest repeatability in total distance covered in the arena (HPD 0.37–0.56), time spent near the walls (HPD 0.28–0.48) and scratching (HPD 0.38–0.56; Table 1).

Phenotypic Differences in Behaviour

The PCA returned five principal components describing variability of behaviour of which we chose the first three with eigenvalue >1, which together explained 74% of the total variance of the data (Table 2). To describe principal components, we used behavioural traits with loadings greater than 0.6 (as absolute value). In the first component, total distance covered in the arena and time spent near the walls were positively correlated, whereas scratching was opposite to them. We interpreted this component as proactive behaviour. The second component was described only by grooming

Table 1
Repeatability (r) of behavioural traits

Behavioural trait	Agreement r	DIC	Adjusted r	DIC	Separated r		DIC
Distance covered in arena	0.35	1540.52	0.43	1449.01	FR	0.31	1447.58
					NR	0.49	
					PR	0.39	
Time near walls	0.24	1633.32	0.33	1473.78	FR	0.27	1474.16
					NR	0.37	
					PR	0.29	
Scratching	0.34	1541.48	0.40	1414.91	FR	0.32	1414.31
					NR	0.48	
					PR	0.39	
Climbing	0.30	1571.64	0.40	1453.18	FR	0.54	1452.44
					NR	0.33	
					PR	0.46	
Grooming	0.19	1650.70	0.22	1607.43	FR	0.37	1604.17
					NR	0.17	
					PR	0.17	
Rearing	0.23	1625.51	0.26	1611.46	FR	0.26	1611.84
					NR	0.25	
					PR	0.18	

Agreement repeatability is repeatability due to differences between individuals, adjusted repeatability is repeatability adjusted for fixed factors and separated repeatability is repeatability separated for different phenotypes. DIC: deviance information criterion; FR: full responders; NR: nonresponders; PR: partial responders.

Table 2
Principal component analysis of behavioural traits measured in the open field test

Variable	Proactive behaviour	Anxiety	High stress response
Distance covered in arena	**0.74**	0.36	0.03
Time near walls	**0.87**	−0.02	0.00
Scratching	**−0.89**	−0.21	−0.16
Climbing	0.25	−0.25	**0.65**
Grooming	0.00	**0.93**	0.01
Rearing	−0.09	0.20	**0.82**
Loadings	2.16	1.14	1.11
Explained variance	0.36	0.19	0.19
Cumulative variance	0.36	0.55	0.74

The table presents only the first three principal components, for which eigenvalues > 1. Loadings > 0.6 (as absolute value) are shown in bold.

and it was considered as anxiety. The third component, which we considered to be a high stress response, described climbing and rearing (Table 2). According to the DIC, the best model explaining the variance of proactive behaviour included phenotype, season, sex, trial and the interactions of phenotype and season, and trial and season, whereas anxiety and high stress response were best described by models that included only trial and season as fixed factors. According to the selection criteria, we conservatively accepted models with the lowest DIC; however, the small difference between DIC for the best model and for the competitive models (ΔDIC < 2) indicates that other models could be equally likely in explaining behaviour (Table 3, Appendix Tables A1 and A2). PC scores were repeatable with $r = 0.45$ (HPD 0.35–0.51) for proactive behaviour, $r = 0.27$ (HPD 0.17–0.34) for anxiety and $r = 0.33$ (HPD 0.23–0.40) for high stress response. Phenotypes did not differ in proactive behaviour (relative to HR: NR: HPD -0.41–0.32, pMCMC = 0.41; R: HPD -0.32–0.53, pMCMC = 0.39), anxiety (relative to HR: NR: HPD -0.39–0.36, pMCMC = 0.18; R: HPD -0.29–0.58, pMCMC = 0.23) or high stress response (relative to HR: NR: HPD -0.32–0.58, pMCMC = 0.55; R: HPD -0.24–0.80, pMCMC = 0.35). Proactive behaviour increased during winter (relative to summer: HPD 2.65–4.03, pMCMC < 0.001) and this increase was lower in responding hamsters than in nonresponders and partial responders (HPD −0.77 − −0.003, pMCMC = 0.05). Anxiety increased in winter relative to summer (HPD 1.03–1.57, pMCMC = 0.004) and decreased from first to second trials in both seasons (HPD −0.55 − −0.032, pMCMC = 0.004). High stress response decreased from summer to winter (HPD −1.60 − −1.06, pMCMC < 0.001) and was greater during second than first trials within both seasons (HPD 0.29–0.53, pMCMC = 0.001).

Seasonal Changes in BMR and m_b

Phenotypes differed in m_b (GLM: $F_{2,\ 308} = 19.08$, $P < 0.001$). Nonresponding animals weighed on average 29.87 ± 0.22 g and were significantly heavier than other phenotypes. Full responders weighed 27.29 ± 0.38 g and partial responders weighed 28.33 ± 0.45 g; this difference was not significant (Tukey's HSD: $P = 0.135$). With acclimation to winter all animals lost on average 4.35 g (GLM: $F_{1,\ 308} = 109.94$, $P < 0.001$). Phenotypes differed in seasonal decrease in m_b (GLM: $F_{2,\ 308} = 8.1$, $P<0.001$). The largest decrease in m_b (ca. 20%) was observed in fully responding animals (from 30.31 ± 0.53 g at the end of summer-like acclimation to 24.27 ± 0.52 g after ca. 16–17 weeks of winter-like acclimation) and the smallest (ca. 8.5%) in nonresponding individuals (from 31.20 ± 0.31 g in summer to 28.55 ± 0.31 g in winter). The m_b of partially responding hamsters decreased from 30.50 ± 0.65 g to 26.15 ± 0.62 g, respectively (by 14%). Overall, males were significantly heavier than females (GLM: $F_{1,\ 308} = 317.11$, $P < 0.001$).

The BMR correlated significantly with m_b (GLM: $F_{1,\ 308} = 359.85$, $P < 0.001$) and increased in winter (GLM: $F_{1,\ 308} = 15.22$, $P < 0.001$). After adjusting for m_b, in summer, BMR was 233.86 ± 1.78 mW and in winter 244.50 ± 1.87mW. Phenotypes differed in BMR (GLM: $F_{2,\ 308} = 6.24$, $P = 0.002$) and in its seasonal change (GLM: $F_{2,308} = 7.96$, $P < 0.001$; Fig. 1). BMR increased from 232.66 ± 1.87 mW in summer to 252.36 ± 3.06 mW in winter in nonresponding animals, and from 235.57 ± 3.73 mW in summer to 247.87 ± 3.63 mW in winter in partially responding animals. The BMR of fully responding animals was 233.32 ± 2.26 mW and did not differ between seasons (Fig. 1). Among fully responding hamsters females had higher BMR than males (239.14 ± 3.05 mW and 227.50 ± 3.50, respectively) while in nonresponding and partially responding hamsters BMR did not differ between the sexes (interaction sex*phenotype: GLM: $F_{2,308} = 4.52$, $P = 0.012$).

Correlation Between Behaviour and Metabolism

The best bivariate models describing the relationship between behaviour and BMR included only three fixed effects: season for both traits, trial for proactive behaviour and m_b for BMR (Table A2).

Table 3
Among- and within-individual correlations between behavioural traits and basal metabolic rate (BMR)

Behavioural traits	DIC	Phenotype	BMR			BMR		
			r_{ind}	95% HDP interval		r_e	95% HDP interval	
				Lower	Upper		Lower	Upper
All phenotypes								
Proactive behaviour	96.65	–	−0.027	−0.142	0.160	−0.034	−0.119	0.062
Anxiety	201.24	–	−0.002	−0.164	0.172	−0.013	−0.082	0.102
High stress response	155.36	–	−0.017	−0.169	0.159	−0.018	−0.099	0.084
Variance separated according to phenotype								
Proactive behaviour	160.37	FR	−0.007	−0.378	0.303	−0.035	−0.201	0.164
		NR	−0.029	−0.196	0.199	−0.027	−0.143	0.088
		PR	−0.068	−0.384	0.354	−0.009	−0.229	0.230
Anxiety	312.05	FR	0.032	−0.322	0.339	−0.025	−0.180	0.172
		NR	0.031	−0.197	0.211	−0.004	−0.107	0.122
		PR	−0.038	−0.371	0.390	0.044	−0.201	0.235
High stress response	211.84	FR	0.047	−0.320	0.348	0.012	−0.210	0.177
		NR	0.021	−0.206	0.195	−0.001	−0.119	0.104
		PR	0.016	−0.413	0.357	0.042	−0.228	0.237

Correlations were calculated for models without separated variance (all phenotypes) and for models with variance separated according to phenotype (FR: full responders; NR: nonresponders; PR: partial responders). DIC: deviance information criteria; HPD: 95% highest posterior densities intervals; r_{ind}: among-individual correlation coefficient; r_e: within-individual correlation coefficient.

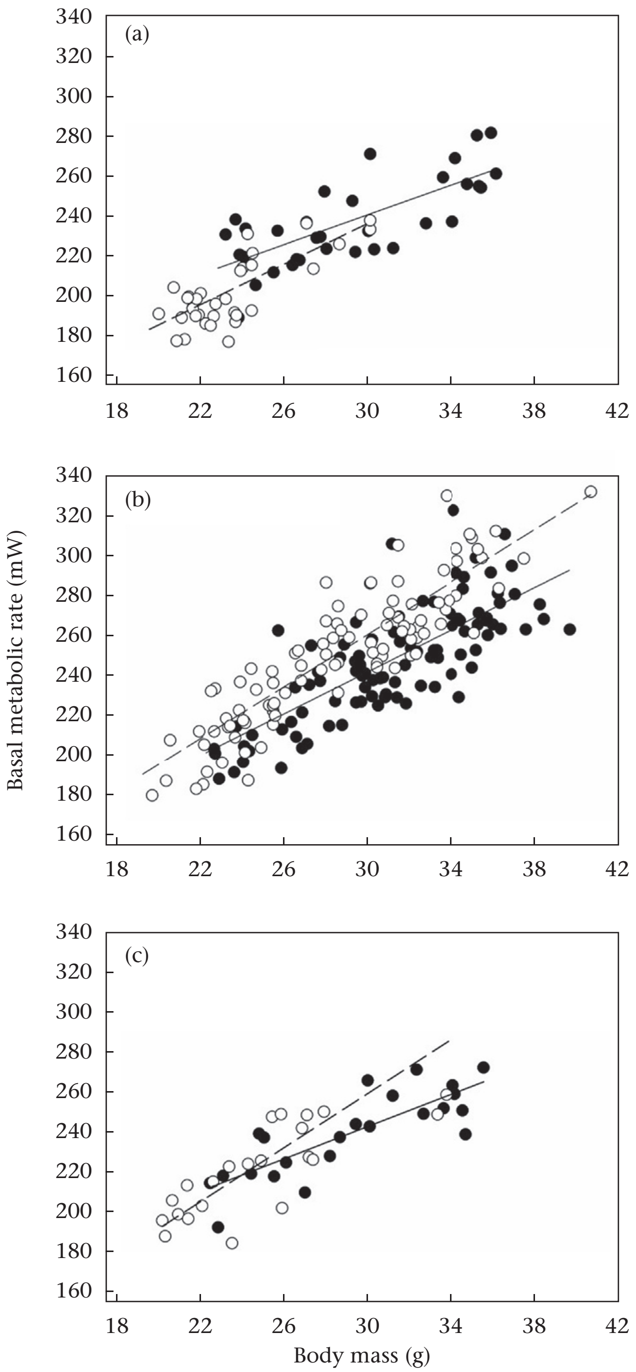

Figure 1. Basal metabolic rate (BMR) of Siberian hamsters (a) fully responding, (b) nonresponding and (c) partially responding to a short photoperiod in winter. Summer BMR: filled circles and solid regression line; winter BMR: open circles and dashed regression line.

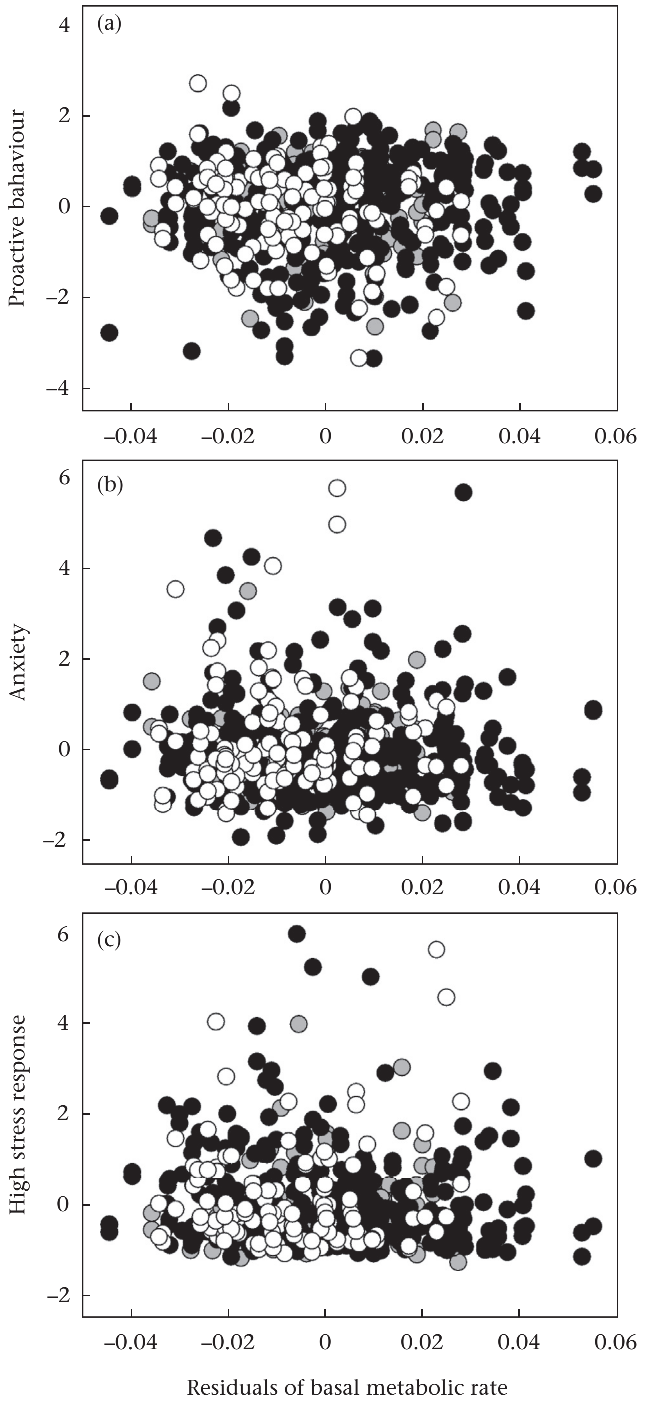

Figure 2. Correlation between principal component scores for behavioural traits and residuals of basal metabolic rate in full responders (open circles), nonresponders (black circles) and partial responders (grey circles). (a) Proactive behaviour, (b) anxiety and (c) high stress response).

We found no correlations between behavioural traits and BMR in any of the phenotypes (Table 3, Fig. 2).

DISCUSSION

Differences in personality may reflect genetic differences, phenotypic plasticity or flexibility (changes in behaviour resulting from environmental change), or all of these (Wolf & Weissing, 2012). The maintenance of different personalities in a population, which are shaped during ontogeny by the interaction between genes and environment, ensures its survival in the face of changing environments (Armitage, 1986; Réale et al., 2007; Dingemanse, Kazem, Réale, & Wright, 2010; Bijleveld et al., 2014). We hypothesized that winter phenotypes of Siberian hamster, fully responding, partially responding or nonresponding to a short photoperiod, represent different personalities. Our results did not support this hypothesis. In all hamsters, proactive behaviour and grooming increased from summer to winter while the stress response decreased. This seasonal difference in behaviour probably reflects a general winter increase in aggression (Jasnow, Huhman, Bartness, & Demas, 2000; Demas, Polacek, Durazzo, & Jasnow, 2004; Scotti, Belén, Jackson, & Demas, 2008; Rendon, Rudolph, Sengelaub, & Demas, 2015). Short-day aggression is independent of adrenocortical hormones (Scotti, Rendon, Greives, Romeo, & Demas, 2015) or gonadal steroids (Caldwell, Glickman, & Smith, 1984; Scotti, Place, & Demas, 2007; 2008). It is related not to reproduction but to harsh environmental conditions and limited resources (Jasnow et al., 2000). However, the seasonal increase in proactive behaviour that we recorded in our study was lowest in the fully responding phenotype indicating its behaviour was less flexible than that of partial responders and nonresponders. This supports our previous findings that the fully responding phenotype is characterized by less flexible energetics than the nonresponding one (Boratyński, Jefimow, & Wojciechowski, 2017). Behavioural traits measured in the open field were repeatable (Table 1) and thus we can conclude that individual Siberian hamsters have different personalities, although these differences did not correlate with differences in winter phenotype. In line with this, Kanda, Louon, and Straley (2012) found that personality traits in Siberian hamsters are heritable and activity is the most repeatable and reliable trait describing personality. Various personalities have been reported for a wide range of species, both homoeothermic and heterothermic, for example wild house mice, *Mus musculus* (Benus et al., 1991; Sluyter, Bult, Lynch, van Oortmerssen, & Koolhaas, 1995; Constantini, Carere, Caramashi, & Koolhaas, 2008), rats, *Rattus norvegicus* (Koolhaas et al., 1999), eastern chipmunks, *Tamias striatus* (Martin & Réale, 2008; Boyer, Réale, Marmet, Pisanu, & Chapuis, 2010; Montiglio, Garant, Pelletier, & Réale, 2012), many bird species (Kurvers et al., 2009; Herborn, Coffey, Larcombe, Alexander, & Arnold, 2011; Jacobs et al., 2014; Trompf & Brown, 2014), reptiles (Le Galliard, Paquet, Cisel, & Montes- Poloni, 2013), numerous invertebrates (reviewed by Kralj-Fiser & Schuett, 2014) and many other taxa (reviewed by Gosling, 2001). Several studies have focused on the relationship between personality and cognition (Griffin, Guillette, & Healy, 2015), personality and speciation (Ingley & Johnson, 2014), personality and sociality (Dingemanse & Araya-Ajoy, 2015; Koski & Burkart, 2015; Öst, Seltmann, & Jaatinen, 2015), personality and immunology (Dosmann, Brooks, & Mateo, 2015) and personality and energy metabolism (Careau et al., 2015). The correlation between energetics and personality may be described by four different models: performance, allocation, independent and substitution (Careau et al., 2008; Careau & Garland, 2012). We predicted that full responders would have lower BMR in winter than nonresponders and partial responders. Indeed, while nonresponders and partial responders increased their BMR from summer to winter, full responders did not change it (Fig. 1). This finding supports the performance model which assumes that animals investing more energy in activity have higher maintenance costs, which ultimately leads to an increase in BMR and to high daily energy expenditure (DEE; Careau et al., 2008; Careau & Garland, 2012). However, a survey of the literature shows that the correlation between BMR and personality is equivocal. Lantová, Zub, Koskela, Šíchová, and Borowski (2011) did not find a clear and significant correlation between personality and BMR in root voles, *Microtus oeconomus*, and only a weak correlation was found in nonreproductive females. Nevertheless, traits characterizing proactive behaviour best explained BMR variability (Lantová et al., 2011). In a 3-year study, Bouwhuis, Quinn, Sheldon, and Verhulst (2014) found a weak but negative correlation between exploratory behaviour and BMR in free-living great tits, *Parus major*, and only in females. The most exploratory females had a 4.5% lower BMR than the least exploratory birds. Similarly, comparison of 19 muroid species also revealed that interspecific variation in exploratory behaviour was negatively correlated with BMR (Careau, Binida-Emonds, Thomas, Réale, & Humphries, 2009). These results would support the allocation but not the performance model for a correlation between BMR and personality. Conversely, other studies, including ours, have supported the performance model. Gębczyński and Konarzewski (2009) reported that mice selected for high BMR had higher voluntary activity than mice from nonselected, control lines. Biro and Stamps (2010) found a positive relationship between personality traits (mainly measured as activity and aggressiveness) and resting metabolic rate (RMR) in 20 of 27 case studies analysed (ranging from invertebrates to mammals). Also, Careau and Garland (2012) found a positive relationship between personality traits and measures of MR (BMR, RMR and standard MR) in nine of 21 case studies of fish and rodents. Thus, the correlation between BMR and personality is still ambiguous. According to the performance model, variation in personality is caused by variation in the size of metabolic organs. This relation is predicted to be positive, that is, individuals with large organs would be more exploratory, more aggressive and bolder to acquire enough energy to sustain the organs (Bijleveld et al., 2014; Careau et al., 2008). We did not measure the mass of organs, but nonresponding animals were the heaviest. More importantly, phenotypes differed in the seasonal change in m_b. From summer to winter, full responders decreased their m_b by ca. 6 g, partial responders by ca. 4.3 g and nonresponders only by ca. 2.6 g. The lowest winter decrease in m_b in nonresponders would also support the performance model. All hamsters were housed under the same conditions (photoperiod, T_a, cage type, food and water availability) so any behavioural or physiological differences between phenotypes are of endogenous, not exogenous origin.

Together, the largest decrease in $m_{b,}$ the smallest seasonal changes in proactive behaviour and lack of winter increase in BMR make fully responding animals better adapted to winter than nonresponding or partially responding phenotypes. However, despite those potential energetic benefits of being full responders, animals of different winter phenotypes coexist in one population and respond to selective pressures depending on the environment (Nelson, 1987; Prendergast et al., 2001). Explicitly, we propose that different winter phenotypes are maintained in the population because they are beneficial in certain environmental conditions. We suggest that nonresponsiveness as an ecological strategy can be advantageous under certain conditions, that is, when energy resources are readily available in winter. If so, nonresponders can gain more food and remain normothermic during the day. In this way they would have a larger DEE, but they would avoid the physiological and ecological costs of torpor (Radzicki, Hejduk, & Bańbura, 1999; but see ; Stawski & Geiser, 2010; Daan, Barnes, & Strijkstra, 1991; Millesi, Prossinger, Dittami, & Fieder, 2001; but see ; Ruczyński & Siemers, 2011; Bouma, Carey, & Kroese, 2009; Humphries, Kramer, & Thomas, 2003; Munro & Thomas, 2004). Thus, nonresponders, which avoid torpor, would be advantageous in an environment of lower energy demands and high resource availability. This environment would be different to that in which heterothermy evolved. Conversely, full responders, which regularly enter torpor, would be favoured in an environment of higher energy demands (e.g. lower ambient temperature and/or less snow cover) and limited resources. They may be less exposed to predators, would spend less energy on activity and would have a smaller DEE. Partial responders would represent an intermediate phenotype. We suggest that within a population there is a continuum not only in torpor use but also in other morphological and physiological traits of the winter phenotype, which, however, are not related to animal personality. This continuum, unlike a simple bimodal distribution, allows for population maintenance despite environmental conditions that change over short and long timescales.

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References

Anchordoquy, H. C., & Lynch, G. R. (2000). Evidence of an annual rhythm in a small proportion of Siberian hamsters exposed to chronic short days. *Journal of Biological Rhythms, 15*, 122–125.

Armitage, K. B. (1986). Individuality, social behavior, and reproductive success in yellow-bellied marmots. *Ecology, 67*, 1186–1193.

Benus, R. F., Bohus, B., Koolhaas, J. M., & van Oortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia, 47*, 1008–1019.

Bernard, D. J., Losee-Olson, S., & Turek, F. W. (1997). Age-related changes in the photoperiodic response of Siberian hamsters. *Biology of Reproduction, 57*, 172–177.

Bijleveld, A. I., Massourakis, G., van der Marel, A., Dekinga, A., Spaans, B., van Gils, J. A., et al. (2014). Personality drives physiological adjustments and is not related to survival. *Proceedings of the Royal Society B, 281*, 20133135 https://doi.org/10.1098/rspb.2013.3135.

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution, 25*, 653–659.

Boratyński, J. S., Jefimow, M., & Wojciechowski, M. S. (2017). Individual differences in the phenotypic flexibility of basal metabolic rate in Siberian hamsters are consistent on short- and long-term timescales. *Physiological & Biochemical Zoology, 90*, 139–152.

Bouma, H. R., Carey, H. V., & Kroese, F. G. M. (2009). Hibernation: The immune system at rest? *Journal of Leukocyte Biology, 88*, 619–624.

Bouwhuis, S., Quinn, J. L., Sheldon, B. C., & Verhulst, S. (2014). Personality and basal metabolic rate in a wild bird population. *Oikos, 123*, 56–62.

Boyer, N., Réale, D., Marmet, J., Pisanu, B., & Chapuis, J. L. (2010). Space use and tick load in an introduced population of Siberian chipmunks *Tamias sibricus*. *Journal of Animal Ecology, 79*, 538–547.

Boyles, J. G., Thompson, A. B., McKechnie, A. E., Malan, E., Humphries, M. M., & Careau, V. (2013). A global heterothermic continuum in mammals. *Global Ecology & Biogeography, 22*, 1029–1039.

Broussard, D. R., Robertson, J. L., Evans, T. E., Faucher, G. K., Semanik, M. G., & Heideman, P. D. (2009). Age-related decline in reproductive sensitivity to inhibition by short photoperiod in *Peromyscus leucopus*. *Journal of Mammalogy, 90*, 32–39.

Caldwell, G. S., Glickman, S. E., & Smith, E. R. (1984). Seasonal aggression independent of seasonal testosterone in wood rats. *Proceedings of the National Academy of Sciences USA, 81*, 5255–5257.

Careau, V., Binida-Emonds, O. R. P., Thomas, D. W., Réale, D., & Humphries, M. M. (2009). Exploration strategies map along fast-slow metabolic and life-history continua in muroid rodents. *Functional Ecology, 23*, 150–156.

Careau, V., & Garland, T. (2012). Personality, and energetics: Correlation, causation, and mechanism. *Physiological & Biochemical Zoology, 85*, 543–571.

Careau, V., Montiglio, P.-O., Garant, D., Pelletier, F., Speakman, J. R., Humphries, M. M., et al. (2015). Energy expenditure and personality in wild chipmunks. *Behavioral Ecology & Sociobiology, 69*, 653–661.

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos, 117*, 641–653.

Constantini, D., Carere, C., Caramashi, D., & Koolhaas, J. M. (2008). Aggressive and non-aggressive personalities differ in oxidative status in selected lines of mice (*Mus musculus*). *Biology Letters, 4*, 119–122.

Daan, S., Barnes, B. M., & Strijkstra, A. M. (1991). Warming up for sleep? Ground squirrels sleep during arousals from hibernation. *Neuroscience Letters, 128*, 265–268.

Demas, G. E., Polacek, K. M., Durazzo, A., & Jasnow, A. M. (2004). Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*). *Hormones & Behavior, 46*, 582–591.

Diedrich, V., Bank, J. H., Scherbarth, F., & Steinlechner, S. (2015). Torpor expression in juvenile and adult Djungarian hamsters (*Phodopus sungorus*) differs in frequency, duration and onset in response to a daily cycle in ambient temperature. *Journal of Thermal Biology, 53*, 23–32.

Dingemanse, N. J., & Araya-Ajoy, Y. G. (2015). Interacting personalities: Behavioural ecology meets quantitative genetics. *Trends in Ecology & Evolution, 30*, 88–97.

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *Journal of Animal Ecology, 82*, 39–54.

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution, 25*, 81–89.

Dosmann, A., Brooks, K. C., & Mateo, J. M. (2015). Evidence for a mechanism of phenotypic integration of behaviour and innate immunity in a wild rodent: Implications for animal personality and ecological immunology. *Animal Behaviour, 101*, 179–189.

Ellison, G. T. H. (1993). Evidence of climatic adaptation in spontaneous torpor among Pouched mice *Saccostomus campestris* from southern Africa. *Acta Theriologica, 38*, 49–59.

Figala, J., Hoffmann, K., & Goldau, G. (1973). Zur Jahresperiodik beim Dsungarischen Zwerghamster *Phodopus sungorus* Pallas. *Oecologia (Berlin), 12*, 89–118.

Freeman, D. A., & Goldman, B. D. (1997a). Photoperiod nonresponsive Siberian hamsters: Effect of age on the probability of nonresponsiveness. *Journal of Biological Rhythms, 12*, 110–121.

Freeman, D. A., & Goldman, B. D. (1997b). Evidence that circadian system mediates photoperiodic nonresponsiveness in Siberian hamsters: The effect of running wheel access on photoperiodic responsiveness. *Journal of Biological Rhythms, 12*, 121–132.

Gębczyński, A. K., & Konarzewski, M. (2009). Locomotor activity of mice divergently selected for basal metabolic rate: A test of hypotheses on the evolution of endothermy. *Journal of Evolutionary Biology, 22*, 1212–1220.

Geiser, F., & Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds—physiological variables and classification of torpor patterns. *Physiological Zoology, 6*, 935–966.

Goldman, S. L., Dhandapani, K., & Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *Journal of Biological Rhythms, 15*, 417–428.

Goldman, S. L., & Goldman, B. D. (2003). Early photoperiod history and short-day responsiveness in Siberian hamsters. *Journal of Experimental Zoology, 296A*, 38–45.

Gorman, M. R., & Zucker, I. (1997). Environmental induction of photo-nonresponsiveness in the Siberian hamster, *Phodopus sungorus*. *American Journal of Physiology, 272*, R887–R895.

Gosling, S. D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin, 127*, 45–86.

Griffin, A. S., Guillette, L. M., & Healy, S. D. (2015). Cognition and personality: An analysis of an emerging field. *Trends in Ecology & Evolution, 30*, 207–214.

Gutowski, J. P., Wojciechowski, M. S., & Jefimow, M. (2011). Diet affects resting, but not basal metabolic rate of normothermic Siberian hamsters acclimated to winter. *Comparative Biochemistry & Physiology A, 160*, 516–523.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software, 33*, 1–22.

Hadfield, J. (2017). *MCMCglmm course notes*. http://cran.us.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf.

Heath, H. W., & Lynch, G. R. (1983). Intraspecific differences in the use of photoperiod and temperature as environmental cues in *Peromyscus leucopus*. *Physiological Zoology, 56*, 289–295.

Heldmaier, G., & Lynch, G. R. (1986). Pineal involvement in thermoregulation and Acclimatization. *Pineal Research Reviews, 4*, 97–139.

Heldmaier, G., & Steinlechner, S. (1981a). Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. *Journal of Comparative Physiology, 142*, 429–437.

Heldmaier, G., & Steinlechner, S. (1981b). Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*. *Oecologia (Berlin), 48*, 265–270.

Herborn, K. A., Coffey, J., Larcombe, S. D., Alexander, L., & Arnold, K. E. (2011). Oxidative profile varies with personality in European greenfinches. *Journal of Experimental Biology, 214*, 1732–1739.

Hoffmann, K. (1973). The influence of photoperiod and melatonin on testis size, body weight, and pelage colour in the Djungarian hamster *Phodopus sungorus*. *Journal of Comparative Physiology, 85*, 267–282.

Humphries, M. M., Kramer, D. L., & Thomas, D. W. (2003). The role of energy availability in mammalian hibernation: An experimental test in free-ranging eastern chipmunks. *Physiological & Biochemical Zoology, 76*, 180–186.

Ingley, S. J., & Johnson, J. B. (2014). Animal personality as a driver of reproductive isolation. *Trends in Ecology & Evolution, 29*, 369–371.

Jacobs, C. G. C., van Overveld, T., Careau, V., Matthysen, E., Adriaensen, F., & Slabbekoorn, H. (2014). Personality-dependent response to field playback in great tits: Slow explorers can be strong responders. *Animal Behaviour, 90*, 65–71.

Jasnow, A. M., Huhman, K. L., Bartness, T. J., & Demas, G. E. (2000). Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). *Hormones & Behavior, 38*, 102–110.

Kaiser, H. F. (1991). Coefficient alpha for a principal component and the kaiser-guttman rule. *Psychological Reports, 68*, 855–858.

Kanda, L. L., Louon, L., & Straley, K. (2012). Stability in activity and boldness across time and context in captive Siberian dwarf hamsters. *Ethology, 118*, 518–533.

Kaseloo, P. A., Crowell, M. G., & Heideman, P. D. (2014). Heritable variation in reaction norms of metabolism and activity across temperatures in a wild-derived population of white-footed mice (*Peromyscus leucopus*). *Journal of Comparative Physiology B, 184*, 525–534.

Kelley, K., Maxwell, S. E., & Rausch, J. R. (2003). Obtaining power or obtaining precision. Delineating methods of sample-size planning. *Evaluation & the Health Professions, 26*, 258–287.

Kerbeshian, M. C., Bronson, F. H., & Bellis, E. D. (1994). Variation in reproductive photoresponsiveness in a wild population of meadow voles. *Biology of Reproduction, 50*, 745–750.

Kliman, R. M., & Lynch, G. R. (1992). Evidence for genetic variation in the occurrence of the photoresponse of the Djungarian hamster, *Phodopus sungorus*. *Journal of Biological Rhythms, 7*, 161–173.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., et al. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews, 23*, 925–935.

Koski, S. E., & Burkart, J. M. (2015). Common marmosets show social plasticity and group-level similarity in personality. *Science Reports, 5*, 8878.

Kralj-Fišer, S., & Schuett, M. (2014). Studying personality variation in invertebrates: Why bother? *Animal Behaviour, 91*, 41–52.

Kurvers, R. H. J. M., Eijkelenkamp, B., van Oers, K., van Lith, B., van Wieren, S. E., Ydenberg, R. C., et al. (2009). Personality differences explain leadership in barnacle geese. *Animal Behaviour, 78*, 447–453.

Lantová, P., Zub, K., Koskela, E., Šíchová, K., & Borowski, Z. (2011). Is there a linkage between metabolism and personality in small mammals? The root vole (*Microtus oeconomus*) example. *Physiology & Behavior, 104*, 378–383.

Larkin, J. E., Freeman, D. A., & Zucker, I. (2001). Low ambient temperature accelerates short-day responses in Siberian hamsters by altering responsiveness to melatonin. *Journal of Biological Rhythms, 16*, 76–86.

Le Galliard, J.-F., Paquet, M., Cisel, M., & Montes-Poloni, L. (2013). Personality and the pace-of-life syndrome: Variation and selection on exploration, metabolism and locomotor performance. *Functional Ecology, 27*, 136–144.

Lighton, J. R. B. (2008). *Measuring metabolic rates*. New York, NY: Oxford University Press.

Lighton, J. R. B., Bartholomew, G. A., & Feener, D. H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiological Zoology, 60*, 524–537.

Lynch, G. R. (1973). Effect of simultaneous exposure to differences in photoperiod and temperature on the seasonal molt and reproductive system of the white-footed mouse, *Peromyscus leucopus*. *Comparative Biochemistry & Physiology A, 44*, 1373–1376.

Lynch, G. R., Lynch, C. B., & Kliman, R. M. (1989). Genetic analyses of photoresponsiveness in the Djungarian hamster, *Phodopus sungorus*. *Journal of Comparative Physiology A, 164*, 475–481.

Lynch, G. R., & Puchalski, W. (1986). Effect of prolonged short day exposure on thermoregulation in the Djungarian hamster *Phodopus sungorus*. In H. C. Heller (Ed.), *Living in the cold: Physiological and biochemical adaptations* (pp. 317–322). New York, NY: Elsevier.

Martin, J. G. A., & Réale, D. (2008). Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. *Animal Behaviour, 75*, 309–318.

Millesi, E., Prossinger, H., Dittami, J. P., & Fieder, M. (2001). Hibernation effects on memory in European ground squirrels (*Spermophilus citellus*). *Journal of Biological Rhythms, 16*, 264–271.

Moffatt, C. A., DeVries, A. C., & Nelson, R. J. (1993). Winter adaptations of male deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*) that vary in reproductive responsiveness to photoperiod. *Journal of Biological Rhythms, 8*, 221–232.

Montiglio, P.-O., Garant, D., Pelletier, F., & Réale, D. (2012). Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunk, *Tamias striatus*. *Animal Behaviour, 84*, 1071–1079.

Munro, D., & Thomas, D. W. (2004). The role of polyunsaturated fatty acids in the expression of torpor by mammals: A review. *Zoology, 107*, 29–48.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews of the Cambridge Philosophical Society, 85*, 935–956.

Nelson, R. J. (1987). Photoperiod-Nonresponsive morphs: A possible variable in microtine population-density fluctuations. *The American Naturalist, 130*, 350–369.

Öst, M., Seltmann, M. W., & Jaatinen, K. (2015). Personality, body condition and breeding experience drive sociality in a facultatively social bird. *Animal Behaviour, 100*, 166–173.

Prendergast, B. J., Kriegsfeld, L. J., & Nelson, R. J. (2001). Photoperiodic polyphenisms in rodents: Neuroendocrine mechanisms, costs, and functions. *The Quarterly Review of Biology, 76*, 293–325.

Przybylska, A. S., Wojciechowski, M. S., Drobniak, S., & Jefimow, M. (2017, January). *Photo-responding hamsters support the allocation model of the relationship between energy metabolism and activity. Oral presentation at the annual meeting of society for integrative and comparative biology*. New Orleans, LA http://www.sicb.org/meetings/2017/SICB2017Abstracts.pdf.

Puchalski, W., & Lynch, G. R. (1986). Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. *Journal of Comparative Physiology A, 159*, 7–11.

Radzicki, G., Hejduk, J., & Bańbura, J. (1999). Tits (Parus major and Parus caerulus) preying upon hibernating bats. *Ornis Fennica, 76*, 93–94.

Raiche, G. (2010). *nFactors: An R package for parallel analysis and non-graphical solutions to the Cattell scree test*. R package version 2.3.3 https://cran.r-project.org/web/packages/nFactors/nFactors.pdf.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B, 365*, 4051–4063.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*, 291–318.

Rendon, N. M., Rudolph, L. M., Sengelaub, D. R., & Demas, G. E. (2015). The agonistic adrenal: Melatonin elicits female aggression via regulation of adrenal androgens. *Proceedings of the Royal Society B, 282*, 20152080.

Roche, D. G., Careau, V., & Binning, S. A. (2016). Demystifying animal 'personality' (or not): Why individual variation matters to experimental biologists. *Journal of Experimental Biology, 219*, 3832–3843.

Ruczyński, I., & Siemers, B. M. (2011). Hibernation does not affect memory retention in bats. *Biology Letters, 7*, 153–155.

Ruf, T., & Heldmaier, G. (1992). The impact of daily torpor on energy requirements in the Djungarian hamster, *Phodopus sungorus*. *Physiological Zoology, 65*, 994–1010.

Ruf, T., & Heldmaier, G. (2000). Djungarian hamsters: Small graminivores with daily torpor. In S. Halle, & N. C. Stenseth (Eds.), *Activity patterns in small mammals. An Ecological approach. Ecological studies 141* (pp. 217–234). Berlin, Germany: Springer-Verlag.

Ruf, T., Stieglitz, A., Steinlechner, S., Blank, J. L., & Heldmaier, G. (1993). Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (*Phodopus sungorus*). *Journal of Experimental Zoology, 267*, 104–112.

Rusak, B., & Zucker, I. (1975). Biological rhythms and animal behavior. *Annual Review of Psychology, 26*, 137–171.

Scotti, M.-A. L., Belén, J., Jackson, J. E., & Demas, G. E. (2008). The role of androgens in the mediation of seasonal territorial aggression in male Siberian hamsters (*Phodopus sungorus*). *Physiology & Behavior, 95*, 633–640.

Scotti, M.-A. L., Place, N. J., & Demas, G. E. (2007). Short-day increases in aggression are independent of circulating gonadal steroids in female Siberian hamsters (*Phodopus sungorus*). *Hormones & Behavior, 52*, 183–190.

Scotti, M.-A. L., Rendon, N. M., Greives, T. J., Romeo, R. D., & Demas, G. E. (2015). Short-day aggression is independent of changes in cortisol or glucocorticoid receptors in male Siberian hamsters (*Phodopus sungorus*). *Journal of Experimental Zoology, 323A*, 331–342.

Sluyter, F., Bult, A., Lynch, C. B., van Oortmerssen, G. A., & Koolhaas, J. M. (1995). A comparison between house mouse lines selected for attack latency or nest-building: Evidence for a genetic basis of alternative behavioral strategies. *Behavior Genetics, 25*, 247–252.

Stawski, C., & Geiser, F. (2010). Seasonality of torpor patterns and physiological variables of a free-ranging subtropical bat. *Journal of Experimental Biology, 213*, 393–399.

Trompf, L., & Brown, C. (2014). Personality affects learning and trade-offs between private and social information in guppies, *Poecilia reticulata*. *Animal Behaviour, 88*, 99–106.

Tucker, V. A. (1965). Oxygen consumption, thermal conductance, and torpor in the California pocket mouse, *Perognathus californicus*. *Journal of Cellular & Comparative Physiology, 65*, 393–403.

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psvchological Bulletin, 83*, 482–504.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology & Evolution, 27*, 452–461.

Appendix

Table A1
Deviance information criteria (DIC) of the univariate models with different numbers of fixed factors

Model	*k*	DIC	ΔDIC
Proactive behaviour			
Phenotype + season + sex + trial + phenotype*season + trial*season	**8**	**1406.52**	0.00
Phenotype + season + sex + trial + trial*season	7	1407.16	0.64
Phenotype + season + sex + trial + phenotype*season + trial*season + season*sex	9	1407.37	0.85
Phenotype + season + sex + trial + phenotype*sex + Phenotype*season + trial*season + season*sex	10	1408.05	1.53
Season + trial	4	1453.22	46.70
Phenotype + season + sex + trial	6	1454.13	47.61
Phenotype + season + trial	5	1454.34	47.82
Anxiety			
Season + trial	**4**	**1603.45**	0.00
Phenotype + season + trial	5	1604.33	0.88
Phenotype + season + sex + trial	6	1604.92	1.47
Phenotype + season + sex + trial + trial*season	7	1605.40	1.95
Phenotype + season + sex + trial + phenotype*season + trial*season	8	1606.23	2.78
Phenotype + season + sex + trial + phenotype*sex + Phenotype*season + trial*season + season * sex	10	1609.40	5.95
Phenotype + season + sex + trial + phenotype*season + trial*season + season * sex	9	1609.67	6.22
High stress response			
Season + trial	**4**	**1506.74**	0.00
Phenotype + season + trial	5	1507.65	0.91
Phenotype + season + sex + trial	6	1508.44	1.70
Phenotype + season + sex + trial + trial*season	7	1510.43	3.69
Phenotype + season + sex + trial + phenotype*season + trial*season + season * sex	9	1511.02	4.28
Phenotype + season + sex + trial + phenotype*season + trial*season	8	1511.83	5.09
Phenotype + season + sex + trial + phenotype*sex + Phenotype*season + trial*season + season * sex	10	1515.44	8.70

Models with the lowest DIC are indicated in bold. *k*: number of parameters in the model; ΔDIC: the difference between the model with the lowest DIC and the current model.

Table A2
Deviance information criteria (DIC) of the bivariate models with different numbers of parameters

Model	Activity and BMR		Anxiety and BMR		High stress response and BMR	
	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC
Sex + phenotype + season + phenotype*season + phenotype*sex + sex*season + trial (for behaviour) + m_{b} (for BMR)	−333.77	12.33	−180.42	15.32	−275.79	15.40
Sex + phenotype + season + phenotype*season + Sex*season + trial (for behaviour) + m_{b} (for BMR)	−334.22	11.88	−180.34	15.40	−277.53	15.32
Sex + phenotype + season + phenotype*season + trial (for behaviour) + m_{b} (for BMR)	−338.65	7.45	−183.84	11.90	−281.90	11.90
Sex + phenotype + season + trial (for behaviour) + m_{b} (for BMR)	−344.49	1.61	−193.21	2.53	−289.85	2.53
Sex + season + trial (for behaviour) + m_{b} (for BMR)	−346.10	0.08	−194.73	1.01	−291.71	1.01
Season + trial (for behaviour) + m_{b} (for BMR)	**−346.02**	**0.00**	**−195.74**	**0.00**	**−292.52**	0.00
[Season + trial (for behaviour) + m_{b} (for BMR)] with separated variation	160.37	506.47	312.05	507.79	211.84	507.79

Model with the lowest DIC is indicated in bold. BMR: basal metabolic rate; ΔDIC: the difference between the model with the lowest DIC and the current model.

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Photoresponsiveness affects life history traits but not oxidative status in a seasonal rodent

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Abstract

Background: Shortening photoperiod triggers seasonal adjustments like cessation of reproduction, molting and heterothermy. However there is a considerable among-individual variation in photoresponsiveness within one population. Although seasonal adjustments are considered beneficial to winter survival, and natural selection should favor the individuals responding to changes in photoperiod (responders), the phenotype non-responding to changes in day length is maintained in population. Assuming the same resource availability for both phenotypes which differ in strategy of winter survival, we hypothesized that they should differ in life history traits. To test this we compared reproductive traits of two extreme phenotypes of Siberian hamster *Phodopus sungorus* – responding and non-responding to seasonal changes in photoperiod. We bred individuals of the same phenotype and measured time to first parturition, time interval between litters, offspring body mass 3, 10 and 18 days after birth and their growth rate. We also analyzed nest-building behavior. Additionally, we estimated the correlation between reproduction, and basal metabolic rate (BMR) and oxidative status in both phenotypes to infer about the effect of reproductive output on future investments in somatic maintenance.

Results: Prior to reproduction responding individuals were smaller than non-responding ones, but this difference disappeared after reproduction. Responding pairs commenced breeding later than non-responding ones but there was no difference in time interval between consecutive litters. Responders delivered smaller offspring than non-responders and more out of responding individuals built the nest during winter than non-responding ones. Reproduction did not affect future investments in somatic maintenance. Phenotypes did not differ in BMR and oxidative status after reproduction. However, concentration of reactive oxygen metabolites (ROM) was highest in responding males, and biological antioxidant potential (BAP) was higher in males of both phenotypes than in females.

Conclusions: Delayed breeding in responding Siberian hamsters and high ROM concentration in male responders support our hypothesis that differences in adjustment to winter result in different life history characteristics which may explain coexistence of both phenotypes in a population. We propose that polymorphism in photoresponsiveness may be beneficial in stochastic environment, where environmental conditions differ between winters. We suggest that non-responding phenotype may be particularly beneficial during mild winter, whereas responders would be favored under harsh conditions. Therefore, none of the phenotypes is impaired when compared to the other.

Keywords: Photoresponsiveness, Polymorphism, Reproduction, Life history traits, Basal metabolic rate, Oxidative stress

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Background

For small Temperate-Zone endothermic animals winter is one of the most energy demanding periods of the year. Low ambient temperature increases energy requirements, whereas food availability is greatly reduced [1, 2]. To survive animals must adjust their physiology and behavior to prevailing environmental conditions. In small mammals these adjustments include gonadal regression and cessation of reproduction, decrease of body mass (m_b), and molt to a winter coat, all to ensure better thermal insulation and lower energy expenditure. Moreover, heterothermic species use torpor which grants additional energy savings [3–5]. Above changes in physiology and behavior are triggered by shortening day [6–8], and ultimately increase the probability to survive winter and reproduce in spring. However, there is a considerable variation in photoresponsiveness among individuals of the same species, or even within the same population [9, 10]. Some individuals do not respond to changes in photoperiod (non-responding phenotype or non-responders), do not regress gonads, do not enter torpor, and do not change m_b, what may lead to higher energy expenditure and eventually to higher costs of winter survival [6, 8, 11, 12]. The proportion of non-responding individuals varies between species and may reach up to 80% in Prairie voles *Microtus ochrogaster* [13], 47% in Turkish hamsters *Mesocricetus brandti* [14], 50% in white footed mice *Peromyscus leucopus* [15] and 25% in deer mice *Peromyscus maniculatus* [16]. Despite the lack of direct evidence for higher survival of responding individuals (responders), previous studies showed that both, daily and seasonal torpor as well as reduction of normothermic body temperature may increase the life span by decreasing risk of predation [17, 18], reducing the overall cost of survival under challenging environmental conditions [17–19] or by reducing the rate of senescence [20–24]. Moreover, cessing reproduction in winter delays reproductive ageing by deceleration of the attrition of ovarian follicles [25], increase of litter size and decrease of the number of failed pregnancies [26]. If seasonal adjustments in physiology (including heterothermy) and behavior are considered beneficial to winter survival and reproductive success, natural selection should favor responding phenotype (responders), eventually leading to elimination of the non-responding one. Nevertheless, both phenotypes are maintained in populations [10, 27]. Place and Cruickshank [25] suggested, that individuals which respond to short photoperiod should have higher reproductive success than non-responding ones. However, to the best of our knowledge this prediction has not been experimentally verified. We hypothesized that winter phenotypes which differ in their strategy of managing energy resources, should also differ in life history traits. In line with that we predicted that different winter phenotypes are associated with different characteristics of life history traits, and that responding individuals commence reproduction later and deliver smaller litters with bigger offspring than non-responding ones. To verify it, and ultimately to test our hypothesis, we used Siberian hamster *Phodopus sungorus* which is highly seasonal rodent, but with a considerable among-individual variation in photoresponsiveness. On average, depending on a population, between 20 and 60% of individuals do not respond to short photoperiod and remain in their summer status throughout the year [28–32]. We used hamsters that were kept in pairs composed of individuals of the same phenotype (responders or non-responders) and measured time to first parturition, m_b of offspring and their growth rate in two consecutive litters. Additionally, we analyzed nest-building behavior as a trait that may differ between responders and non-responders, and which may be associated with differences in life history traits.

To inquire about the consequences of reproduction on future somatic maintenance, we analyzed parameters of oxidative stress and antioxidant defense in post-reproductive males and females. Imbalance between reactive oxygen species production and antioxidant defense leads to oxidative stress [33–36], which reflects the cost of somatic maintenance [37]. Since reproduction increases energy expenditure, it was hypothesized that it may lead to increased production of reactive oxygen species and eventually launch the protective mechanisms that limit oxidative damage [38–40]. High costs of antioxidant defense may in turn reduce further investment into somatic maintenance leading to oxidative stress [38, 41, 42]. However, available data on oxidative cost of reproduction are ambiguous, or even contradictory. On the one hand, it was reported that reproductive animals had increased oxidative stress (OS) [38, 43] and decreased antioxidant capacity (AC) [41, 42, 44, 45]. On the other hand, pregnancy and lactation was found to protect against OS [40, 46–48]. Other studies showed negative correlation between life history traits such as litter size or litter mass and OS [38, 49], and positive correlation between clutch size and AC [50]. Assuming that the type of response to winter may affect life history traits and therefore investment into reproduction, we predicted that responders and non-responders differ in their investment in somatic maintenance what would be reflected in different oxidative status after reproduction. Because in most mammals cost of reproduction for males is restricted to mate competition [51, 52], and in Siberian hamsters sire presence in the nest does not affect pup survival and development [53, 54], we also predicted that dams, which bear most of the reproduction cost, invest less into somatic maintenance after reproduction and may be a subject to oxidative stress. To test these predictions, we measured basal metabolic rate (BMR), OS and AC in reproductive and non-reproductive individuals of both phenotypes.

Results

Life history traits in two phenotypes of Siberian hamster

All hamsters gained body mass during breeding season (LME: $F_{(1,\ 103.59)} = 69.99$, $P < 0.001$), and m_{b} of males and females increased by 10 and 17%, respectively (Table 1). Responders differed in m_{b} from non-responders only before breeding (LME: $F_{(1,\ 103.59)} = 15.37$, $P < 0.001$), when responding individuals were smaller than non-responding ones (Table 1). There was also a significant interaction between reproductive status and sex (LME: $F_{(1,\ 111.70)} = 18.20$, $P < 0.001$) and reproducing females weighted more than non-reproducing ones, while in males this relation was opposite (Table 2).

Litter size did not differ between consecutive litters (LME: $F_{(1,\ 38)} = 0.02$, $P = 0.88$; Table 1) and it ranged between one and eight pups. Also phenotypes did not differ in litter size (LME: $F_{(1,\ 38)} = 2.18$, $P = 0.15$; Table 1).

Body mass of individual offspring was negatively related to litter size (LME: $F_{(1,\ 44.84)} = 4.68$, $P = 0.04$). Independent of the parental phenotype, mean m_{b} of individual pup increased with consecutive litters (LME: $F_{(1,\ 107.41)} = 4.11$, $P = 0.04$). Offspring m_{b} increased with age (LME: $F_{(1,\ 93.54)} = 2156.80$, $P < 0.001$), but responding pairs delivered smaller offspring than non-responding ones (LME: $F_{(1,\ 48.46)} = 5.97$, $P = 0.002$; Table 1, Fig. 1 a-c). The growth rate did not differ between phenotypes (LME: $F_{(1,\ 93.54)} = 0.84$, $P = 0.36$) resulting in smaller m_{b} at weaning in offspring of responders (Fig. 1 d).

Phenotypes differed in the time of commencing breeding ($U = 20.00$, $P = 0.008$) but not in the time interval between consecutive litters ($U = 20.00$, $P = 0.12$; Table 1). In both phenotypes dam m_{b} correlated neither with the time of first parturition (NR: $r = -0.65$, N = 8, $P = 0.08$; R: $r = 0.31$, N = 9, $P = 0.41$) nor with the time interval between litters (NR: $r = 0.02$, N = 8, $P = 0.97$; R: $r = -0.28$, N = 9, $P = 0.47$). However, among responders, bigger sires bred earlier than smaller ones ($r = -0.71$, N = 12, $P = 0.01$) whereas there was no such relationship among non-responders ($r = 0.26$, N = 10, $P = 0.47$; Fig. 2). There was also no correlation between sire m_{b} and time interval between consecutive litters (NR: $r = -0.17$, N = 8, $P = 0.69$; R: $r = -0.21$, N = 9, $P = 0.58$).

Nest-building behavior

Both the proportion of using the paper tube as well as the propensity to build the nest were repeatable ($r = 0.46$, 95% CI 0.40–0.52, $P < 0.001$; and $r = 0.25$, 95% CI 0.10–0.38, $P = 0.05$; respectively). In winter both phenotypes increased the use of paper tube (LME: $F_{(1,\ 218)} = 83.10$, $P < 0.001$) and the propensity to build the nest ($\chi^2_{(1,\ 296)} = 46.91$, $P < 0.001$), however, in responding individuals this increase was greater than in non-responding

Table 1 Comparison of life-history traits between winter phenotypes of Siberian hamster

		Phenotype	
Life history trait		responding	non-responding
m_{b} before breeding (g)	males	26.81 ± 3.74	32.66 ± 3.56 [a]
	females	23.04 ± 2.11	24.97 ± 2.61 [a]
m_{b} after breeding (g)	males	32.79 ± 3.37	34.13 ± 3.62
	females	30.02 ± 4.30	28.22 ± 3.32
litter size	1st	5.33 ± 1.61	4.20 ± 2.04
	2nd	5.11 ± 2.20	4.60 ± 1.51
offspring m_{b} (g)	3 days	2.56 ± 0.31	2.94 ± 0.40 [a]
	10 days	6.43 ± 0.82	6.97 ± 0.92 [a]
	18 days	12.65 ± 1.85	13.45 ± 1.79 [a]
growth rate (g day^{-1})	1st	0.65 ± 0.12	0.69 ± 0.11
	2nd	0.71 ± 0.11	0.71 ± 0.11
time of commencing breeding (days)		36.50 ± 3.56	22.00 ± 2.24 [a]
time interval between consecutive litters (days)		27.00 ± 5.28	38.50 ± 4.92
degree of use of paper tube %	winter	87.10 ± 12.04	77.78 ± 16.49 [a]
	summer	66.74 ± 20.24 [b]	67.67 ± 24.72 [b]
propensity to build the nest %	winter	91.30	72.50 [a]
	summer	30.40 [b]	43.10 [b]

Values are mean ± SD or median ± SE in case of time of commencement to breeding and time interval between consecutive litters

[a] difference between phenotypes; $P \leq 0.05$

[b] difference between seasons within phenotype; $P \leq 0.05$

Table 2 Comparison of life-history traits between breeding and non-breeding Siberian hamsters

		Phenotype	
Life history trait		breeding	non-breeding
m_b before breeding (g)	males	30.17 ± 4.54	31.95 ± 4.36
	females	24.72 ± 2.71	23.95 ± 2.44
m_b after breeding (g)	males	32.37 ± 2.62	35.87 ± 3.87 [a]
	females	30.12 ± 3.42	26.65 ± 3.03 [a]
degree of use of paper tube %	winter	84.20 ± 12.81	74.55 ± 18.57
	summer	68.82 ± 20.86	64.02 ± 27.14
propensity to build the nest %	winter	88.3	61.1 [a]
	summer	43.6 [b]	31.5 [b]

Values are mean ± SD

[a] difference between reproductive status; $P \leq 0.05$

[b] difference between seasons within reproductive status; $P \leq 0.05$

ones (degree of using paper tube: LME: $F_{(1,\ 218)} = 10.61$, $P = 0.001$; propensity to build the nest: $\chi^2_{(1,\ 148)} = 6.58$, $P = 0.01$, Table 1). Non-breeding individuals tended to use paper tubes slightly less than breeding ones (LME: $F_{(1,\ 70)} = 2.99$, $P = 0.09$). Breeding and non-breeding hamsters differed in their propensity to build the nest only in winter ($\chi^2_{(1,\ 148)} = 14.96$, $P < 0.001$), while in summer, the percentage of hamsters building the nest was similar (Table 2).

BMR and oxidative status

Mean parental BMR equaled 0.27 ± 0.01 W and correlated with hamster m_b (GLM: $F_{(1,\ 81)} = 6.59$, $P = 0.012$). After adjusting for m_b, BMR did not differ between reproducing and non-reproducing individuals (GLM: $F_{(1,\ 81)} = 2.17$, $P = 0.14$), between sexes (GLM: $F_{(1,\ 81)} = 0.01$, $P = 0.93$) or between phenotypes (GLM: $F_{(1,\ 81)} = 0.19$, $P = 0.66$; Fig. 3, Table 3).

Concentration of ROM did not differ between reproducing and non-reproducing hamsters (GLM: $F_{(1,\ 70)} = 0.36$, $P = 0.55$), but it differed between phenotypes (GLM: $F_{(1,\ 70)} = 6.80$, $P = 0.01$) and sexes (GLM: $F_{(1,\ 70)} = 6.60$, $P = 0.01$; Fig. 4 a). Responders had higher concentration of ROM than non-responders and in males it was higher than in females. These differences most probably resulted from high concentration of ROM in responding males (GLM: $F_{(1,\ 70)} = 6.24$, $P = 0.01$; Table 3). Biological antioxidant potential did not differ between hamsters of different reproductive status (GLM: $F_{(1,\ 70)} = 1.37$, $P = 0.25$) or between phenotypes (GLM: $F_{(1,\ 70)} = 0.01$, $P = 0.96$; Fig. 4 b). However, there was an effect of sex on BAP (GLM: $F_{(1,\ 70)} = 5.32$, $P = 0.02$), and in males

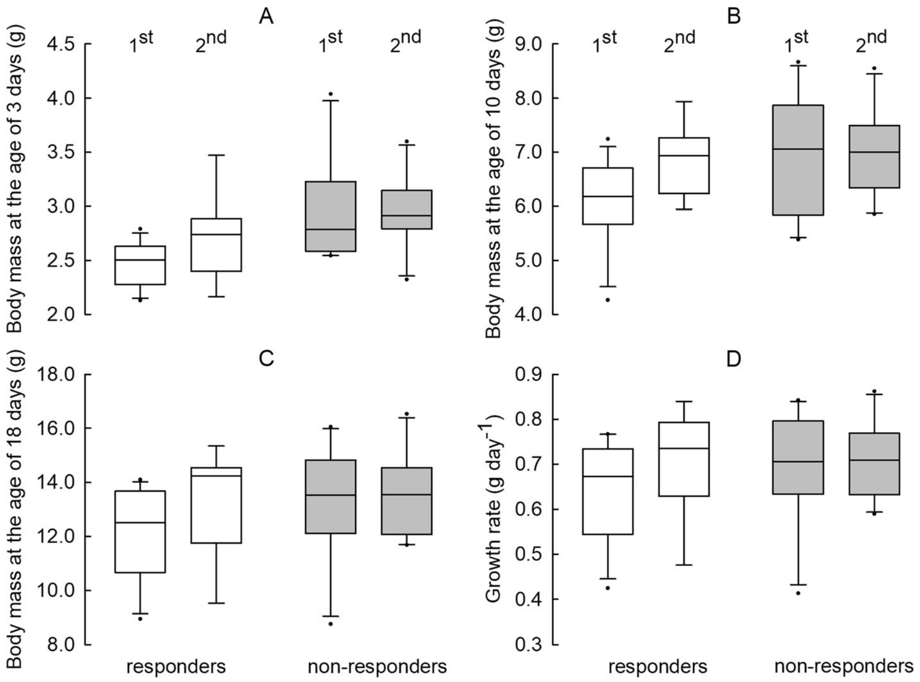

Fig. 1 Life history traits (**a**: body mass at the age of 3 days; **b**: body mass at the age of 10 days; **c**: body mass at the age of 18 days; **d**: mean daily growth rate between 3rd and 18th day of life) in responding and non-responding pairs. Ordinal numbers above figures indicate consecutive litters. Lines inside boxes indicate median, while boxes cover the 25th to 75th percentiles. Whiskers indicate maximum value below upper fence and minimum value above lower fence. Dots indicate outliers

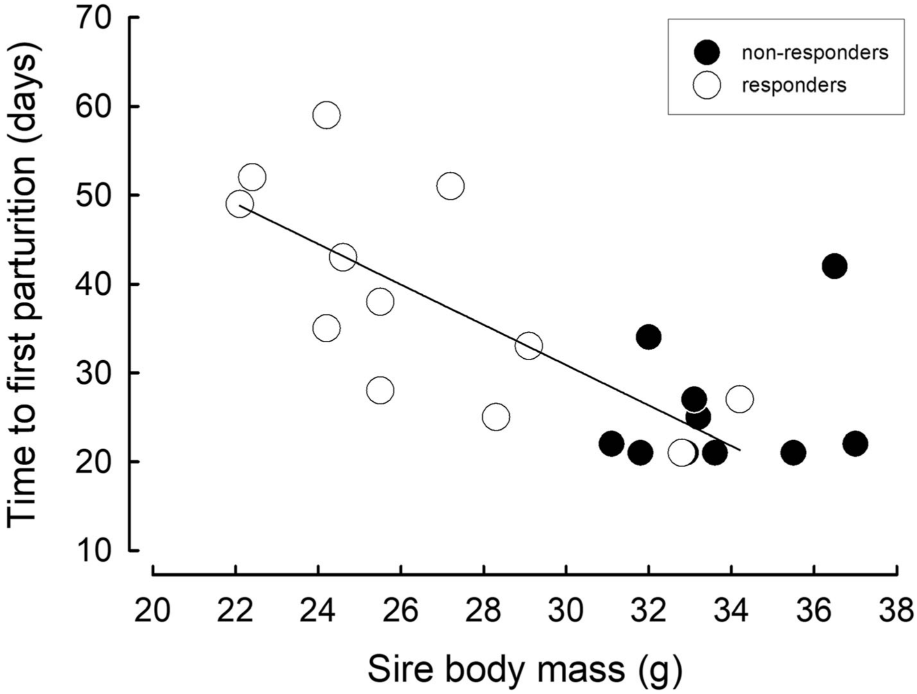

Fig. 2 Relationship between sire body mass and time to first parturition in responding and non-responding hamsters. Regression line ($y = -2.27\ x + 99.00$, $r^2 = 0.50$, $F_{(1,\ 10)} = 10.03$, $P = 0.01$) indicates relationship between sire body mass and time to the first parturition in responding hamsters

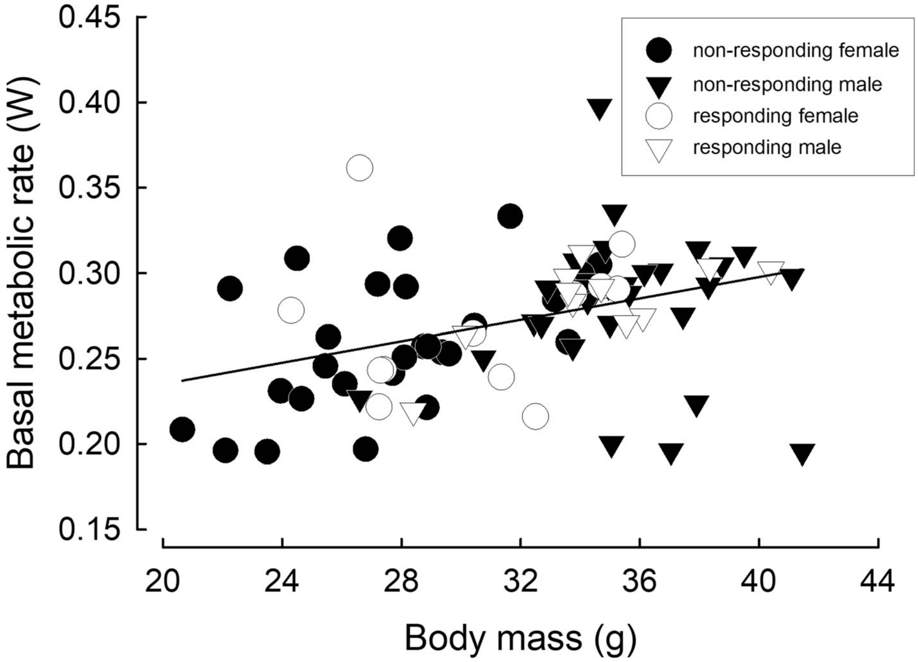

Fig. 3 Relationship between basal metabolic rate and body mass ($y = 0.003\ x + 0.17$, $r^2 = 0.14$, $F_{(1,\ 79)} = 13.38$, $P < 0.001$) in responding and non-responding Siberian hamsters

Table 3 Comparison of basal metabolic rate and oxidative status between winter phenotypes of Siberian hamster

		Phenotype	
Life history trait		responding	non-responding
Basal metabolic rate (W)		268 ± 0.009	273 ± 0.005
Reactive Oxygen Metabolites (mg dL^{-1})	male	15.50 ± 8.00	9.71 ± 1.54 [a]
	female	10.77 ± 1.88 [b]	9.67 ± 2.91
Biological Antioxidant Potential (μM Vit-C L^{-1})	male	3525.98 ± 360.47	3596.77 ± 237.59
	female	3517.56 ± 655.83 [b]	3230.47 ± 608.63 [b]

Values are mean ± SD or marginal mean ± SE in case of Basal metabolic rate
[a] difference between reproductive status; $P < 0.05$
[b] difference between sexes within phenotype; $P < 0.05$

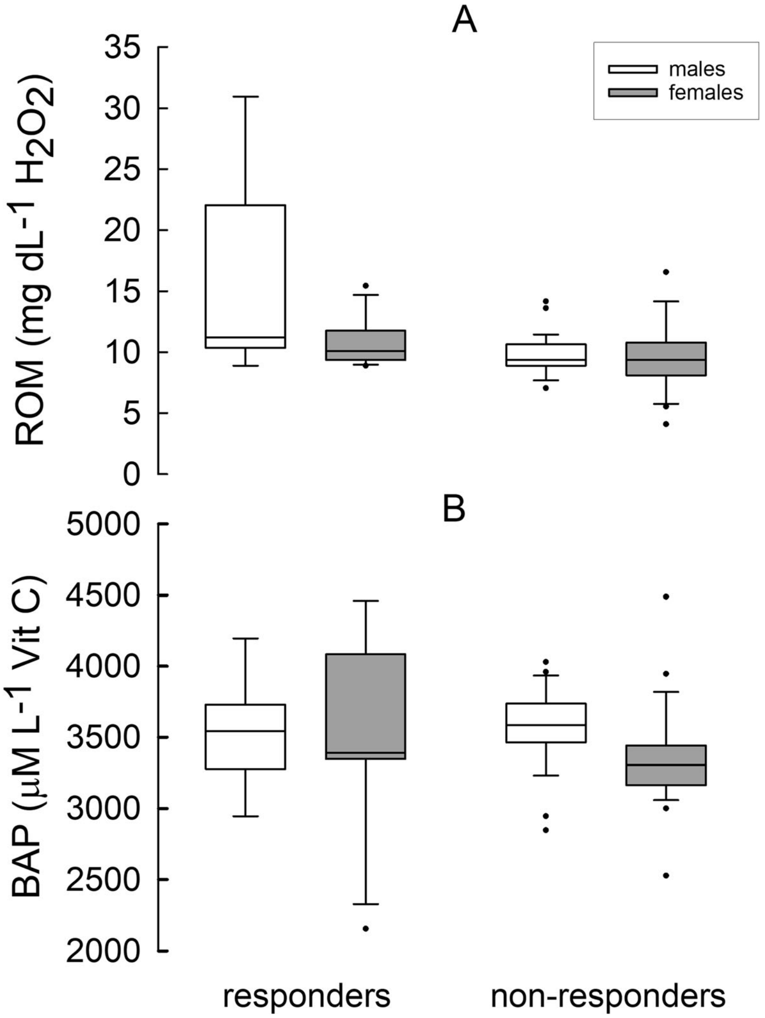

Fig. 4 a: Reactive oxygen metabolites (ROM) and **b**: biological antioxidant potential (BAP) in plasma in responding and non-responding Siberian hamsters. Boxes cover the 25th to 75th percentiles, lines inside boxes indicate median, while whiskers indicate maximum value below upper fence and minimum value above lower fence. Dots indicate outliers of both phenotypes BAP was higher than in females (Table 3).

Discussion

In small mammals of the Temperate-Zone seasonal adjustments, including gonadal regression, molting to winter coat and heterothermy reduce energy expenditure in winter. Previous studies suggested that individuals which respond to winter photoperiod may have longer life span, and higher reproductive success than non-responding ones [17, 21, 25, 55]. Here we aimed to test the hypothesis that different strategies of surviving winter are related to differences in life history traits. To do so, we compared reproductive characteristics of two phenotypes of Siberian hamsters – responding and non-responding to seasonal changes in day length. We also analyzed the relationship between reproduction, energy metabolism, and oxidative status in this species.

Effect of phenotype on life history traits

Out of the studied population we created 11 pairs of responding and 29 pairs of non-responding hamsters. All pairs of responders bred successfully, while among non-responders 17 pairs did not breed at all. A possible explanation for that could be different rate of aging in both phenotypes. Previous studies which showed delayed reproductive ageing in individuals maintained under short photoperiod provide indirect support for this hypothesis [25, 26]. Place and Cruickshank [25] suggested that non-responders age faster than responders and found that female Siberian hamsters responding to short days had greater number of ovarian primordial follicles at older age than non-responding ones. Our animals were about one year old when paired. In a closely related species, the Djungarian hamster *Phodopus campbelli*, symptoms of reproductive aging occured already in 6-month old animals, and in 8-month-olds fertility (delivery success) and fecundity (litter size and weaning success) were reduced by half [56]. Thus, it is possible that our non-responding individuals were reproductively older than responding ones. One could argue that another reason for not breeding of non-responders was low m_b of non-breeding females. They were ~ 15% lighter than the breeding ones and after winter-like acclimation they increased m_b slower than breeding females (Table 2). However, this explanation seems unlikely because at the same time some of breeding females were as small as non-breeding ones.

We found a clear correlation between photoresponsiveness and time to first reproduction. Individuals of the photo-responding phenotype commenced breeding about two weeks later than non-responding ones (Table 1). Arguably, delayed breeding in responders resulted from gonadal regression in this phenotype [8, 57, 58]. Likewise, in some hibernating species, e.g. golden-mantled ground squirrels *Callospermophilus lateralis* and Richardson's ground squirrels *Urocitellus richardsonii*, males terminate hibernation even a month before females to rebuild gonads and m_b prior to breeding season [59, 60]. In responding Siberian hamsters time of commencement of breeding correlated negatively with sire body mass – smaller responding males required more time to achieve reproductive activity (Fig. 2). Although hamsters may mate on the day of parturition, the second litter is usually delayed [61]. Here, independent of phenotype, most animals delivered second litter within ~ 32 days after delivery of the first one. There was also no difference between responders and non-responders in the litter size (Table 1). In both phenotypes it was ~ 5 pups, that is within the typical range for Siberian hamsters [54, 62, 63]. In the same species Place an co-authors [26] found that dams which responded to short photoperiod produced bigger litters than non-responding ones, but in that study non-responsiveness was induced by pinealectomy. In our study, independent of the parental phenotype, the growth rate of pups was ~ 0.7 g per day which corresponds to a range reported previously for this species ($0.56–0.94\ \text{g day}^{-1}$, depending on ambient temperature and litter size) [54, 61–63]. Because males were in the nests only with the first litters, and the growth rates were similar in both litters, these results support previous findings that in Siberian hamsters presence of a sire does not affect offspring growth rate or pup survival [53].

Despite similar litter size and offspring growth rate, responders delivered smaller pups than non-responders, and this difference was maintained until weaning (Table 1). Offspring mass increased with subsequent litters (Fig. 1a-c), most probably as a result of increasing m_b of dams, which were 15% heavier at the end of reproduction than just before the start of breeding. In Djungarian hamsters offspring mass increased with dam age, whereas litter size decreased [56]. Positive relationship between maternal size and offspring size (MSOS) is common among species, from invertebrates to vertebrates [64, 65]. The source of MSOS correlation is still debatable, however it is possibly related to maternal age, nutritional status and body condition [66]. Most data for the positive effect of sire m_b and paternal care on offspring condition come from avian studies [67–69], but there is also strong evidence for Djungarian hamsters [53, 54, 61, 63, 70–72]. Conversely, in Siberian hamsters the presence of male in the nest is not necessary and does not affect pup survival and development [53].

In individually kept hamsters there were no differences between males and females in nest building behavior (Table 1). During summer acclimation number of animals that built a nest from paper tube supplied to the cage, or the degree of chewing it, did not differ between sexes or phenotypes. However, after acclimation to winter-like conditions among responders more individuals built nests and chew more paper tubes than among non-responders. Apparently, this correlated with smaller m_b of responders, who presumably used their nest to increase thermal insulation.

BMR and oxidative status

Investment into reproduction requires significant increase in metabolism [38, 73, 74]. Female laboratory Swiss mice selected for high BMR produced more milk and presented better parental care, measured as the offspring growth rate, than mice with low BMR [75, 76], whereas eastern chipmunks *Tamias striatus* with higher daily energy expenditure produced bigger litters [74]. However, in C57 mice [77] and in Siberian hamsters (this study) breeding and non-breeding individuals did not differ in BMR. Similarly, there was no difference between responders and non-responders (Table 3). One could argue that this lack of difference was an effect of measuring metabolism even a month after weaning of the last litter. However, McLean and Speakman [78] found that elevated metabolism in female brown long-eared bats *Plecotus auritus* was maintained even 80 days after weaning. Thus, we argue that Siberian hamsters did not increase BMR during reproduction. It agrees with the results showing that reproduction is not demanding for animals in natural environments [42, 44, 79, 80]. In many species dams adjust litter size or offspring mass to their current parental efficiency, therefore only forced increase of reproduction costs (e.g. enlarged litter size) would lead to increased metabolism [81–83].

The theory of increased susceptibility to oxidative stress during pregnancy and lactation has a long history [45, 84], however its experimental support is ambiguous. Increase of metabolism and ROM generation not necessarily result in oxidative stress [40, 46]. In reproducing laboratory mice oxidative damage increased in plasma [49] but decreased in liver [38, 49]. In bank voles *Myodes glareolus* reproduction led even to decrease of oxidative damage to lipids but did not change the oxidative damage to proteins [46]. Moreover, oxidative stress increased with litter size in mice [38] and eastern chipmunks [74], but not in bank voles [46] or canaries *Serinus canaria* [40]. Relationship between antioxidant capacity and reproduction is also equivocal. Reproducing mice showed lower [85], higher [38], or unchanged [48] activity of antioxidant enzymes. Antioxidant capacity increased with number of nestlings in male zebra finches *Taeniopygia guttata,* but decreased in females [41], decreased in both sexes of great tits *Parus major* [42], but did not change in striped hamsters *Cricetulus barabensis* [43].

Oxidative stress may also be understood as a proxy for somatic maintenance – increasing when resources available for somatic maintenance are insufficient [37, 40]. Based on our initial prediction that responders and non-responders differ in life history traits, we expected differences in their oxidative status after reproduction [37, 86]. However, we neither found differences between phenotypes in ROM concentration nor in BAP (Table 3, Fig. 4). We also did not find differences between breeding and non-breeding individuals. Under limited resource availability reproduction comes at a cost of self-maintenance, what may result in lower investment in antioxidant defense and eventually in oxidative stress [33–36, 39, 86, 87]. According to the theory of ageing, the loss of cell function during senescence is triggered by a shift in the redox state of the cell and oxidative damage induced by free radicals [88, 89]. This could result in accumulating deleterious mutations and shortening life span [88, 90]. Indeed, negative relationship between investment in reproduction and longevity was well established ([91–95], but see [96, 97]). In contrast, heterothermy use was considered to extend life span [17, 18, 20–23] and to reduce the extinction risk [19]. Moreover, even a small decrease in body temperature may lead to lower oxidative stress and may increase life span, as in transgenic mice with normothermic body temperature lower by 0.5 °C than in wild-type C57/BL6 mice [24]. Siberian hamsters kept under short days not only enter daily torpor but also decrease their normothermic body temperature by ~ 0.7 °C [98]. However, we found no differences in oxidative status between individuals which were using daily torpor during acclimation to winter-like conditions (responders) and those which were not (non-responders). All individuals had similar ROM and BAP levels, apart from responding males which had ~ 50% higher ROM concentration (Table 3). On the one hand this could result from the higher growth rate after winter-like acclimation in responders than in non-responders, but on the other hand it suggests impaired somatic maintenance in post-reproductive period. Higher ROM concentration only in responding males suggests that their antioxidant defense was less effective than in females, which could have been protected by antioxidant properties of estrogens [99, 100]. Yet, overall, males had higher BAP level than females (Fig. 4), suggesting that despite higher ROM concentration, responding males were not subjected to oxidative stress during reproduction.

Conclusions

To the best of our knowledge this study is the first attempt to compare the reproductive characteristics of phenotypes of the same species which differ in their response to short photoperiod. On the one hand, cessation of reproduction in winter and m_b reduction in responding animals delayed commencement of breeding and resulted in smaller offspring m_b. On the other hand, the number of non-breeding non-responders suggests that they aged faster than responders. Despite above differences in life history characteristics, both phenotypes did not differ in metabolic rate and oxidative status, suggesting no effect of reproduction on future investment in somatic maintenance. Delayed breeding in responding pairs and high post-reproductive ROM concentration in male responders support our hypothesis that differences in the adjustment to winter conditions result in different characteristics of life history traits. Because litter size and growth rate were the same in responders and non-responders, and assuming the same resource availability for both phenotypes, we propose that despite differences in the strategy of managing energy resources in winter, they achieve similar fitness, what may explain coexistence of both phenotypes in the population.

However, here we compared only two extreme phenotypes, responding and non-responding to short photoperiod, whereas within one population of Siberian hamsters we can observe entire spectrum of winter phenotypes [101]. Such polymorphism may be beneficial in stochastic environments, where environmental conditions may differ between winters. We propose that non-responding phenotype may be particularly beneficial during mild winters, in the environment with abundant energy supplies and immediate access to food after winter. Responders would be favored during harsh winters, when energy savings mechanisms (e.g. decrease in m_b, gonadal regression and daily torpor) are the most important phenotypic adjustments. If so, from an evolutionary point of view, none of the phenotypes would be impaired when compared to another and polymorphism would be maintained in population.

Methods

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decisions nos. 3/2015, 31–33/2015, 35/2015).

Animals and housing

We used Siberian hamsters from the outbred colony maintained at the Department of Biology and Environmental Protection at Nicolaus Copernicus University in Toruń, Poland. After birth animals were kept under summer-like conditions, i.e. at ambient temperature (T_a) = 18 ± 2 °C and long photoperiod (16 L:8 D) for 4 months. Then, to induce change to winter phenotype, animals were transferred to winter-like conditions ($T_a = 10 \pm 2$ °C and short photoperiod (8 L:16 D)) for 3 months. After completing the acclimation to winter-like conditions hamsters were transferred to summer-like conditions again and 2 weeks later they were paired for breeding. Animals were fed with standard rodent food (Labofeed, Morawski, Kcynia, Poland) and supplied with drinking water ad libitum.

Defining winter phenotypes

Winter phenotypes were determined based on fur coloration and use of torpor. A hamster was classified as responding if it at least partially changed fur color and entered at least one episode of torpor (subcutaneous temperature; $T_{sc} \leq 32$ °C), and as non-responding if it remained grey and did not enter torpor throughout winter. Animals that showed only one trait of winter phenotype (i.e. only white fur or only torpor) were classified as partial responders [101] and were excluded from analyses. To assess the use of torpor, we monitored individuals' T_{sc} with miniature data loggers. Loggers (model TL3–1-27; accuracy of 0.3 °C between 0 °C and 45 °C, constructed by Dr. Dmitry Petrovski, Russian Academy of Sciences, Novosibirsk, Russia) weighted ~ 0.8 g and allowed downloading data wirelessly. Before implantation, loggers were pre-calibrated against a precise mercury-in-glass thermometer between 15 °C and 40 °C. After ~ 6 weeks of winter acclimation loggers were implanted subcutaneously into the interscapular region under ketamine (40 mg kg^{-1}; Ketamina 10%, Biowet, Puławy, Poland) and xylazine (8 mg kg^{-1}; Sedazin 2%, Biowet, Puławy, Poland) anesthesia. The incisions were closed with absorbable sutures (Safil 5/0, Aesculap AG, Tuttlingen, Germany). After surgery all hamsters were kept in the laboratory for 1 day in individual cages (under short photoperiod but $T_a = 22$ °C) and then were transferred back to the animal facility room. Subcutaneous temperature was recorded every 10 min and downloaded every 2 weeks.

Life history traits

Two weeks after changing conditions to summer-like hamsters (~ 1 year old) were paired into 40 phenotypically-matching pairs composed of responding or non-responding hamsters (11 pairs of responders and 29 pairs of non-responders). All animals were weighed before being paired and also after birth of the second litter (sires) or after weaning of the second litter (dams). Animals remained paired for 14 weeks or until delivery of the second litter. Immediately after birth of the second litter, males were separated from females to prevent further breeding. Eventually, 12 pairs of non-responding hamsters and 11 pairs of responding hamsters bred successfully and delivered at least one litter. Seventeen out of non-responding pairs did not breed at all and these animals were used as a control group in the analyses of the cost of reproduction. Then we determined life history traits associated with reproduction: time of commencement of breeding, time interval between consecutive litters, litter size, mass of litter at 3rd, 10th and 18th day of life and growth rate of offspring. Whole litters were weighed together, to the nearest 0.1 g (SPU402, OHAUS, Parsippany, NJ, USA) and mean m_b of individual offspring was calculated as total mass of the litter divided by number of offspring.

Nest-building behavior

During acclimation to summer- and winter-like conditions, before mating, nest-building behavior tests were done in individual hamsters twice in each season (10 days apart). Toilet paper tubes were used as a nesting material. Paper tubes (~ 5.50 g, ~ 10 cm length and ~ 3 cm in diameter) were dried for 72 h at 50 °C, weighed, and put into the animal's home cage. After 48 h, non-chewed remains of tubes were collected, dried for 72 h, and weighed to calculate the proportion of tube chewed by a hamster. In addition, we analyzed the propensity of animals to build a nest. We did not assess the complexity of nest structure but considered any roundish pile of paper pieces with a visible print of animal's body as a nest.

Oxidative stress and antioxidant capacity

At least 2 weeks, but not later than 6 weeks after weaning of the second litter we measured OS and AC in plasma. In non-reproducing pairs oxidative status was assessed at the same time as in reproducing ones. OS and AC were measured using Free Radical Analytical System (FRAS4 evolvo, H&D, Parma, Italy; henceforth: H&D). This system measured the concentration of reactive oxygen metabolites (ROM) and biological antioxidant potential (BAP) in plasma. ROM concentration was measured with dROM-kit (d-ROM-kit, REDOX Kit; H&D) as a level of total H_2O_2, and BAP was measured using PAT-kit (Plasma Antioxidant Test, REDOX Kit; H&D) as a vitamin C concentration. Blood sample (~ 100 μl) was taken from the retro-orbital sinus by capillary puncture. Immediately after bleeding we applied analgesic eye drops into hamster's eye (Alcaine 5 mg/ml, Alcon Polska, Warsaw, Poland).

Basal metabolic rate

Basal metabolic rate was measured at least 2 weeks, but not later than 4 weeks, after weaning of the last litter or after separation of control pairs by indirect calorimetry using an open-flow respirometry system (Sable Systems International, Las Vegas NV, USA; henceforth: SSI). Gas exchange was measured for ~ 8 h within the thermoneutral zone of Siberian hamsters (at 29 ± 1 °C [102, 103]) and BMR was calculated as the lowest rate of O_2 consumption during 3 min in two last hours of the test. All data were acquired using ExpeData software (SSI) at 0.5 Hz. During measurement animals were sealed in 0.85 L respirometry chambers made of transparent polypropylene food containers (HPL 808, Lock&Lock, Hana Cobi, South Korea) which were placed in a temperature controlled cabinet (ST-1200, Pol-Eko-Aparatura, Wodzisław Śląski, Poland). Because BMR measurement requires post-absorptive conditions, hamsters did not have access to water and food during the measurement. Previously we found that this period was sufficient to ensure post-absorptive conditions in the species [103]. We used two parallel respirometry systems, which allowed to simultaneously measure 14 individuals.

Air was pulled from outside the building using air pump (5HCE-10-M553, Gast Manufacturing, Benton Harbor, MI, USA) and compressed in a balloon. Then air was dried and scrubbed of CO_2 with a PureGas Generator (Puregas, Westminster, CO, USA). The main air stream was then split into chambers and a reference gas stream. Air flow rate (~ 330 mL min^{-1}) was regulated upstream of each respirometry chamber with a precise needle valve. Air streams leaving the respirometry chambers were selected sequentially with a computer-controlled multiplexer (Intelligent Multiplexer V3, SSI) and flow rate was measured downstream with a mass flow meter (FlowBar-4, SSI). After flow measurement, air stream was subsampled at a rate of ~ 100 mL min^{-1} and water vapor pressure was measured (RH-300, SSI., USA). Then air stream was pulled through a nafion dryer tubes (product number 17049, VacuMed, Ventura, CA, USA) embedded in silica gel and finally was dried with magnesium perchlorate (product number 11636.36, VWR International, Gdańsk, Poland). Then concentrations of O_2 and CO_2 were measured. Oxygen consumption rate ($\dot{V}O_2$) and CO_2 production rate ($\dot{V}CO_2$) of seven individuals were measured with FoxBox-C integrated CO_2 and O_2 analyzer (SSI). In the remaining seven hamsters we used FC10a analyzer (SSI) and CA10 analyzer (SSI) to measure $\dot{V}O_2$ and $\dot{V}CO_2$, respectively. Gas exchange of each animal was recorded for 5 min, every 44 min and the baseline gas concentration readings were done every 20 min. Metabolic rate was calculated after Lighton and coauthors [104] as follows:

$$MR\ (W) = \frac{\dot{V}O_2(16 + 5.164 \times RER)}{60}$$

where $\dot{V}O_2$ is the rate of oxygen consumption (ml O_2/min) and $RER = \frac{\dot{V}CO_2}{\dot{V}O_2}$ was calculated from recorded $\dot{V}CO_2$ and $\dot{V}O_2$. All individuals, regardless of phenotype and sex, had similar RER ranging between 0.79 ± 0.03 in summer and 0.83 ± 0.05 in winter.

Statistical analysis

We divided animals into two groups reflecting reproductive status: breeding and non-breeding animals. Animals in the latter group were paired but for unknown reasons did not breed. Only non-responding individuals did not breed.

To analyze whether phenotype and reproductive status affect parental m_b before and after breeding, we used linear mixed model (LME) with animal ID as a random factor, phenotype, sex, reproductive status and time of measurement (before or after breeding) as fixed factors and two interactions: sex × reproductive status and phenotype × time of measurement.

To analyze the effect of phenotype on litter size and offspring m_b, we used LME with pair ID set as a random factor. Consecutive litter number and parental phenotype were used as fixed factors. Additionally, in the model analyzing offspring m_b we used litter size and day of measurement (3rd, 10th or 18th) as a covariates. We used the day of m_b measurement as a continuous variable, to obtain information about the body mass gain (growth rate). Model included also two interactions: parental phenotype × day of measurement and parental phenotype × litter size. We did not include dam and sire m_b in the analysis because of its non-significant effect on litter size (dam: $F_{(1,19.17)} = 0.03$, $P = 0.87$; sire: $F_{(1,19.28)} = 0.10$, $P = 0.76$) and offspring m_b (dam: $F_{(1,18.26)} = 0.46$, $P = 0.50$; sire: $F_{(1,17.99)} = 0.80$, $P = 0.38$).

Because of small sample size and non-normal distribution of data, effect of phenotype on time of commencement of breeding and time interval between consecutive litters were analyzed by Mann-Whitney U test. Medians were compared by Mood's median test. We used Pearson correlation to relate time of commencement of breeding and time interval between consecutive litters with m_b of dams and sires, separately for responders and non-responders.

To analyze the effect of reproduction and phenotype on BMR, ROM concentration and BAP we used General Linear Model (GLM) with phenotype, sex, and reproductive status as fixed factors. In the model analyzing the variability of BMR we used m_b as covariate. The initial models included also interaction of sex and phenotype, but finally, this interaction was excluded, except for the model analyzing the variability of ROM concentration.

Repeatability of the proportion of paper tube chewed by individual was calculated with LME, and repeatability of propensity to build the nest was calculated with Generalized Linear Mixed Model (GLMM) and binary probit model. To test if phenotype and breeding status affect nest-building behavior we used LME with ID as a random factor, sex, phenotype, breeding status, season and trial as fixed factors, and interactions: phenotype × season and season × trial. To test if phenotypes differ in ability to build the nest we used contingency table using Poisson errors. We used four explanatory variables: phenotype, sex, season and breeding status.

All results, except for time of commencement to breeding and time interval between consecutive litters (median ± SE), are presented as mean ± SD. Statistical significance was accepted at $P < 0.05$.

Abbreviations

AC: Antioxidant capacity; BAP: Biological antioxidant potential; BMR: Basal metabolic rate; m_b: body mass; OS: Oxidative stress; ROM: Reactive oxygen metabolites

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ASP - acquisition of data, analysis and interpretation of data, drafting the first draft of the manuscript and the revisions, MSW - concept of the study, acquisition of data, critically revising the manuscript. MJ – concept of the study, acquisition of data, critically revising the manuscript. All authors read and approved the final manuscript.

Ethics approval

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decisions nos. 3/2015, 31–33/2015, 35/2015).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. Scholander PF, Hock R, Walters V, Irving L. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biol Bull. 1950;99(2):259–71.
2. McKechnie AE. Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. J Comp Physiol B. 2008;178(3):235–47.
3. Heldmaier G. Seasonal Acciimatization of energy requirements in mammals: functional significance of body weight control, hypothermia, torpor and hibernation. In: Wieser W, Gnaiger E, editors. Energy transformation in cells and organisms. Stuttgart: Fischer; 1989. p. 130–9.
4. Geiser F. Evolution of daily torpor and hibernation in birds and mammals: importance of body size. Clin Exp Pharmacol Physiol. 1998;25(9):736–9.
5. Ruf T, Geiser F. Daily torpor and hibernation in birds and mammals. Biol Rev. 2015;90(3):891–926.
6. Hoffmann K. The influence of photoperiod and melatonin on testis size, body weight and pelage colour in the Djungarian hamster. J Comp Physiol. 1973;85:267–82.
7. Goldman B, Hall V, Hollister C, Reppert S, Roychoudhury P, Yellon S, et al. Diurnal changes in pineal melatonin content in four rodent species: relationship to photoperiodism. Biol Reprod. 1981;24(4):778–83.

8. Gorman MR, Zucker I. Seasonal adaptations of Siberian hamsters. II. Pattern of change in day length controls annual testicular and body weight rhythms. Biol Reprod. 1995;53:116–25.
9. Heath HW, Lynch GR. Intraspecific differences for melatonin-induced reproductive regression and the seasonal molt in Peromyscus leucopus. Gen Comp Endocrinol. 1982;48(3):289–95.
10. Nelson RJ. Photoperiod-nonresponsive morphs: a possible variable in microtine population-density fluctuations. Am Nat. 1987;130(3):350–69.
11. Nelson RJ. Photoperiodic regulation of reproductive development in male Praire voles: influence of laboratory breeding. Biol Reprod. 1985;33:418–22.
12. Heideman PD, Rightler M, Sharp K. A potential microevolutionary life-history trade-off in white-footed mice (Peromyscus leucopus). Funct Ecol. 2005;19(2):331–6.
13. Nelson RJ. Photoperiod influences reproduction in the prairie vole (Microtus ochrogaster). Biol Reprod. 1985;33(3):596–602.
14. Ogilvie KM, Stetson MH. The timing of gonadal refractoriness in the female Turkish hamster (Mesocricetus brandti) is not dependent on the timing of gonadal regression. Biol Reprod. 1990;42:787–91.
15. Whitaker W. Some effects of artificial illumination on the repro-duction in the white-footed mouse, P. Leucopus noveboracensis. J Exp Zool. 1940;83:33–60.
16. Desjardins C, Lopez MJ. Environmental cues evoke differential responses in pituitary-testicular function in deer mice*. Endocrinology. 1983;112(4):1398–406.
17. Nowack J, Stawski C, Geiser F. More functions of torpor and their roles in a changing world. J Comp Physiol B. 2017;187(5–6):889–97.
18. Austad SN, Fischer KE. Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. J Gerontol. 1991;46(2):B47–53.
19. Geiser F, Turbill C. Hibernation and daily torpor minimize mammalian extinctions. Naturwissenschaften. 2009;96(10):1235–40.
20. Turbill C, Ruf T, Smith S, Bieber C. Seasonal variation in telomere length of a hibernating rodent. Biol Lett. 2013;9(2):20121095.
21. Turbill C, Smith S, Deimel C, Ruf T. Daily torpor is associated with telomere length change over winter in Djungarian hamsters. Biol Lett. 2012;8(2):304–7.
22. Ruf T, Bieber C, Turbill C. Survival, Aging, and Life-History Tactics in Mammalian Hibernators. In: Ruf T, Bieber C, Arnold W, Millesi E, editors. Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 123–32.
23. Wu CW, Storey KB. Life in the cold: links between mammalian hibernation and longevity. Biomol Concepts. 2016;7(1):41–52.
24. Conti B, Sanchez-Alavez M, Winsky-Sommerer R, Morale MC, Lucero J, Brownell S, et al. Transgenic mice with a reduced core body temperature have an increased life span. Science. 2006;314(5800):825–8.
25. Place NJ, Cruickshank J. Graded response to short photoperiod during development and early adulthood in Siberian hamsters and the effects on reproduction as females age. Horm Behav. 2009;55(3):390–7.
26. Place NJ, Tuthill CR, Schoomer EE, Tramontin AD, Zucker I. Short day lengths delay reproductive aging. Biol Reprod. 2004;71(3):987–92.
27. Prendergast BJ, Kriegsfeld LJ, Nelson RJ. Photoperiodic polyphenisms in rodents: neuroendocrine mechanisms, costs, and functions. Q Rev Biol. 2001;76(3):293–325.
28. Lynch GR, Lynch CB, Kliman RM. Genetic analyses of photoresponsiveness in the Djungarian hamster, Phodopus sungorus. J Comp Physiol A. 1989;164(4):475–81.
29. Kliman RM, Lynch GR. Evidence for genetic variation in the occurrence of the photoresponse of the Djungarian hamster, Phodopus sungorus. J Biol Rhythms. 1992;7(2):161–73.
30. Ruf T, Stieglitz A, Steinlechner S, Blank JL, Heldmaier G. Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (Phodopus sungorus). J Exp Zool. 1993;267(2):104–12.
31. Freeman DA, Goldman BD. Photoperiod nonresponsive Siberian hamsters: effect of age on the probability of nonresponsiveness. J Biol Rhythm. 1997; 12(2):110–21.
32. Goldman SL, Dhandapani K, Goldman BD. Genetic and environmental influences on short-day responsiveness in Siberian hamsters (Phodopus sungorus). J Biol Rhythm. 2000;15(5):417–28.
33. Barja de Quiroga G. Brown fat thermogenesis and exercise: two examples of physiological oxidative stress? Free Radic Biol Med. 1992;13(4):325–40.
34. Frisard M, Ravussin E. Energy metabolism and oxidative stress: impact on the metabolic syndrome and the aging process. Endocrine. 2006;29(1):27–32.
35. Isaksson C, Sheldon BC, Uller T. The challenges of integrating oxidative stress into life-history biology. BioScience. 2011;61(3):194–202.
36. Speakman JR, Blount JD, Bronikowski AM, Buffenstein R, Isaksson C, Kirkwood TB, et al. Oxidative stress and life histories: unresolved issues and current needs. Ecol Evol. 2015;5(24):5745–57.
37. Latta LC, Tucker KN, Haney RA. The relationship between oxidative stress, reproduction, and survival in a bdelloid rotifer. BMC Ecol. 2019;19(1):7.
38. Garratt M, Vasilaki A, Stockley P, McArdle F, Jackson M, Hurst JL. Is oxidative stress a physiological cost of reproduction? An experimental test in house mice. Proc R Soc Lond B Biol Sci. 2011;278(1708):1098–106.
39. Selman C, Blount JD, Nussey DH, Speakman JR. Oxidative damage, ageing, and life-history evolution: where now? Trends Ecol Evol. 2012;27(10):570–7.
40. Costantini D, Casasole G, Eens M. Does reproduction protect against oxidative stress? J Exp Biol. 2014;217(Pt 23):4237–43.
41. Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Sorci G. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecol Lett. 2004;7(5):363–8.
42. Losdat S, Helfenstein F, Gaude B, Richner H. Reproductive effort transiently reduces antioxidant capacity in a wild bird. Behav Ecol. 2011;22(6):1218–26.
43. Zhao XY, Zhang JY, Cao J, Zhao ZJ. Oxidative Damage Does Not Occur in Striped Hamsters Raising Natural and Experimentally Increased Litter Size. PLoS ONE. 2015;10(10):e0141604.
44. Wiersma P, Selman C, Speakman JR, Verhulst S. Birds sacrifice oxidative protection for reproduction. Proc R Soc Lond B Biol Sci. 2004;271(Suppl 5): 360–3.
45. Monaghan P, Metcalfe NB, Torres R. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett. 2009;12(1):75–92.
46. Ołdakowski L, Piotrowska Ż, Chrząścik KM, Sadowska ET, Koteja P, Taylor JR. Is reproduction costly? No increase of oxidative damage in breeding bank voles. J Exp Biol. 2012;215(Pt 11):1799–805.
47. Ołdakowski L, Wasiluk A, Sadowska ET, Koteja P, Taylor JR. Reproduction is not costly in terms of oxidative stress. J Exp Biol. 2015;218(Pt 24):3901–10.
48. Vaanholt LM, Milne A, Zheng Y, Hambly C, Mitchell SE, Valencak TG, et al. Oxidative costs of reproduction: oxidative stress in mice fed standard and low antioxidant diets. Physiol Behav. 2016;154:1–7.
49. Stier A, Reichert S, Massemin S, Bize P, Criscuolo F. Constraint and cost of oxidative stress on reproduction: correlative evidence in laboratory mice and review of the literature. Front Zool. 2012;9(1):37.
50. Bize P, Devevey G, Monaghan P, Doligez B, Christe P. Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. Ecology. 2008;89(9):2584–93.
51. Garratt M, McArdle F, Stockley P, Vasilaki A, Beynon RJ, Jackson MJ, et al. Tissue-dependent changes in oxidative damage with male reproductive effort in house mice. Funct Ecol. 2012;26(2):423–33.
52. Michener GR. Sexual differences in reproductive effort of Richardson's ground squirrels. J Mammal. 1998;79(1):1–19.
53. Timonin ME, Wynne-Edwards KE. Neither reduced photoperiod, nor female-related social cues, nor increased maternal thermal stress result in a paternally responsive Phodopus sungorus male. Physiol Behav. 2006;88(4–5):309–16.
54. Wynne-Edwards KE, Lisk RD. Differential effects of paternal presence on pup survival in two species of dwarf hamster (Phodopus sungorus and Phodopus campbelli). Physiol Behav. 1989;45(3):465–9.
55. Turbill C, Bieber C, Ruf T. Hibernation is associated with increased survival and the evolution of slow life histories among mammals. Proc Biol Sci. 2011;278(1723):3355–63.
56. Edwards HE, Tweedie CJ, Terranova PF, Lisk RD, Wynne-Edwards KE. Reproductive aging in the Djungarian hamster, Phodopus campbelli. Biol Reprod. 1998;58(3):842–8.
57. Butler MP, Turner KW, Park JH, Schoomer EE, Zucker I, Gorman MR. Seasonal regulation of reproduction: altered role of melatonin under naturalistic conditions in hamsters. Proc R Soc Lond B Biol Sci. 2010;277(1695):2867–74.
58. Boratyński JS, Jefimow M, Wojciechowski MS. Melatonin attenuates phenotypic flexibility of energy metabolism in a photoresponsive mammal, the Siberian hamster. J Exp Biol. 2017;220(Pt 17):3154–61.
59. Healy JE, Burdett KA, Buck CL, Florant GL. Sex differences in torpor patterns during natural hibernation in golden-mantled ground squirrels (Callospermophilus lateralis). J Mammal. 2012;93(3):751–8.
60. Michener GR. Sexual differences in over-winter torpor patterns of Richardson's ground squirrels in natural hibernacula. Oecologia. 1992;89:397–406.
61. Newkirk KD, McMillan HJ, Wynne-Edwards KE. Length of delay to birth of a second litter in dwarf hamsters (Phodopus): evidence for post-implantation embryonic diapause. J Exp Zool. 1997;278(2):106–14.
62. Newkirk KD, Cheung BL, Scribner SJ, Wynne-Edwards KE. Earlier thermoregulation and consequences for pup growth in the Siberian versus Djungarian dwarf hamster (Phodopus). Physiol Behav. 1998;63(3):435–43.

63. Stulberg SE, Wynne-Edwards KE. Maternal and pup contributions to different patterns of pup growth in Phodopus species. Physiol Behav. 1998; 64(5):715–22.
64. Lim JN, Senior AM, Nakagawa S. Heterogeneity in individual quality and reproductive trade-offs within species. Evolution. 2014;68(8):2306–18.
65. Parker GA, Begon M. Optimal egg size and clutch size - effects of environment and maternal phenotype. Am Nat. 1986;128(4):573–92.
66. Rollinson N, Rowe L. The positive correlation between maternal size and offspring size: fitting pieces of a life-history puzzle. Biol Rev. 2016;91(4): 1134–48.
67. West HE, Capellini I. Male care and life history traits in mammals. Nat Commun. 2016;7:11854.
68. Stockley P, Hobson L. Paternal care and litter size coevolution in mammals. Proc R Soc Lond B Biol Sci. 2016;283(1829):20160140.
69. Przybylo R, Wiggins DA, Merilä J. Breeding success in blue tits: good territories or good parents? J Avian Biol. 2001;32(3):214–8.
70. Wynne-Edwards KE. Biparental care in Djungarian but not Siberian dwarf hamsters (Phodopus). Anim Behav. 1995;50:1571–85.
71. Walton JM, Wynne-Edwards KE. Paternal care reduces maternal hyperthermia in Djungarian hamsters (Phodopus campbelli). Physiol Behav. 1997;63(1):41–7.
72. Jones JS, Wynne-Edwards KE. Paternal hamsters mechanically assist the delivery, consume amniotic fluid and placenta, remove fetal membranes, and provide parental care during the birth process. Horm Behav. 2000;37(2): 116–25.
73. Speakman JR. The physiological costs of reproduction in small mammals. Philos Trans R Soc Lond Ser B Biol Sci. 2008;363(1490):375–98.
74. Bergeron P, Careau V, Humphries MM, Réale D, Speakman JR, Garant D. The energetic and oxidative costs of reproduction in a free-ranging rodent. Funct Ecol. 2011;25(5):1063–71.
75. Sadowska J, Gębczyński AK, Paszko K, Konarzewski M. Milk output and composition in mice divergently selected for basal metabolic rate. J Exp Biol. 2015;218(Pt 2):249–54.
76. Sadowska J, Gębczyński AK, Konarzewski M. Basal metabolic rate is positively correlated with parental investment in laboratory mice. Proc R Soc Lond B Biol Sci. 2013;280(1753):20122576.
77. Johnston SL, Souter DM, Erwin SS, Tolkamp BJ, Yearsley JM, Gordon IJ, et al. Associations between basal metabolic rate and reproductive performance in C57BL/6J mice. J Exp Biol. 2007;210(Pt 1):65–74.
78. McLean JA, Speakman JR. Effects of body mass and reproduction on the basal metabolic rate of brown long-eared bats (Plecotus auritus). Physiol Biochem Zool. 2000;73(1):112–21.
79. Oksanen TA, Jonsson P, Koskela E, Mappes T. Optimal allocation of reproductive effort: manipulation of offspring number and size in the bank vole. Proc R Soc Lond B Biol Sci. 2001;268(1467):661–6.
80. Koivula M, Koskela E, Mappes T, Oksanen TA. Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. Ecology. 2003; 84(2):398–405.
81. König B, Markl H. Maternal care in house mice I. The weaning strategy as a means for parental manipulation of offspring quality. Behav Ecol Sociobiol. 1987;20:1–9.
82. Flajšman K, Jerina K, Pokorny B. Age-related effects of body mass on fertility and litter size in roe deer. PLoS ONE. 2017;12(4):e0175579.
83. Fuchs S. Optimality of parental investment - the influence of nursing on reproductive success of mother and female young house mice. Behav Ecol Sociobiol. 1982;10(1):39–51.
84. Salmon AB, Marx DB, Harshman LG. A cost of reproduction in Drosophila melanogaster: stress susceptibility. Evolution. 2001;55(8):1600–8.
85. Zhang Y, Brasher AL, Park NR, Taylor HA, Kavazis AN, Hood WR. High activity before breeding improves reproductive performance by enhancing mitochondrial function and biogenesis. J Exp Biol. 2018;221(7):jeb177469.
86. Costantini D. Oxidative stress and hormesis in evolutionary ecology and physiology : a marriage between mechanistic and evolutionary approaches Berlin: Springer; 2014. 348 p p.
87. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Phys Regul Integr Comp Phys. 2007;292(1):R18–36.
88. Sohal RS, Orr WC. The redox stress hypothesis of aging. Free Radic Biol Med. 2012;52(3):539–55.
89. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev. 1998;78(2):547–81.
90. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996;273(5271):59–63.
91. Daan S, Deerenberg C, Dijkstra C. Increased daily work precipitates natural death in the kestrel. J Anim Ecol. 1996;65(5):539–44.
92. Snell TW, King CE. Lifespan and fecundity patterns in rotifers: the cost of reproduction. Evolution. 1977;31(4):882–90.
93. Branco AT, Schilling L, Silkaitis K, Dowling DK, Lemos B. Reproductive activity triggers accelerated male mortality and decreases lifespan: genetic and gene expression determinants in Drosophila. Heredity (Edinb). 2017; 118(3):221–8.
94. Hansen M, Flatt T, Aguilaniu H. Reproduction, fat metabolism, and life span: what is the connection? Cell Metab. 2013;17(1):10–9.
95. Tabatabaie V, Atzmon G, Rajpathak SN, Freeman R, Barzilai N, Crandall J. Exceptional longevity is associated with decreased reproduction. Aging-Us. 2011;3(12):1202–5.
96. Tarin JJ, Gomez-Piquer V, Garcia-Palomares S, Garcia-Perez MA, Cano A. Absence of long-term effects of reproduction on longevity in the mouse model. Reprod Biol Endocrinol. 2014;12:84.
97. Ricklefs RE, Cadena CD. Lifespan is unrelated to investment in reproduction in populations of mammals and birds in captivity. Ecol Lett. 2007;10(10):867–72.
98. Heldmaier G, Steinlechner S, Ruf T, Wiesinger H, Klingenspor M. Photoperiod and thermoregulation in vertebrates: body temperature rhythms and thermogenic acclimation. J Biol Rhythm. 1989;4(2):251–65.
99. Borrás C, Sastre J, García-Sala D, Lloret A, Pallardó FV, Viña J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med. 2003;34(5):546–52.
100. Persky AM, Greene P, Stubley L, Howell C, Zaulyanov L, Brazeau GA, Simpkins JW. Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and in vitro. Proc Soc Exp Biol Med. 2008; 232:59–66.
101. Przybylska AS, Wojciechowski MS, Jefimow M. Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behaviour. Anim Behav. 2019;147:105–14.
102. Puchalski W, Bockler H, Heldmaier G. Effect of food deprivtion on thermogenic capacity in the djungarian hamster.pdf; 1983.
103. Gutowski JP, Wojciechowski MS, Jefimow M. Diet affects resting, but not basal metabolic rate of normothermic Siberian hamsters acclimated to winter. Comp Biochem Physiol A Mol Integr Physiol. 2011;160(4):516–23.
104. Lighton JRB, Bartholomew GA, Feener DH. Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant Atta colombica Guer. Physiol Zool. 1987;60(5):524–37.

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RESEARCH **Open Access**

Polymorphism of winter phenotype in Siberian hamster: consecutive litters do not differ in photoresponsiveness but prolonged acclimation to long photoperiod inhibits winter molt

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Abstract

Background: The theory of delayed life history effects assumes that phenotype of adult individual results from environmental conditions experienced at birth and as juvenile. In seasonal environments, being born late in the reproductive season affects timing of puberty, body condition, longevity, and fitness. We hypothesized that late-born individuals are more prone to respond to short photoperiod (SP) than early born ones. We used Siberian hamsters *Phodopus sungorus*, a model species characterized by high polymorphism of winter phenotype. We experimentally distinguished the effect of litter order (first or third) from the effect of exposure to long photoperiod (LP) before winter (3 months or 5 months) by manipulating the duration of LP acclimation in both litters. We predicted that, irrespective of the litter order, individuals exposed to long photoperiod for a short time have less time to gather energy resources and consequently are more prone to developing energy-conserving phenotypes. To assess effect of litter order, duration of acclimation to long days, and phenotype on basal cost of living we measured basal metabolic rate (BMR) of hamsters.

Results: Individuals born in third litters had faster growth rates and were bigger than individuals from first litters, but these differences vanished before transfer to SP. Litter order or duration of LP acclimation had no effects on torpor use or seasonal body mass changes, but prolonged acclimation to LP inhibited winter molting both in first and third litters. Moreover, individuals that did not molt had significantly higher BMR in SP than those which molted to white fur. Although one phenotype usually predominated within a litter, littermates were often heterogeneous. We also found that over 10% of individuals presented late response to short photoperiod.

Conclusions: Our data indicate that duration of postnatal exposure to LP may define propensity to photoresponsiveness, regardless of the litter in which animal was born. Existence of littermates presenting different phenotypes suggests a prudent reproductive strategy of investing into offspring of varied phenotypes, that might be favored depending on environmental conditions. This strategy could have evolved in response to living in stochastic environment.

Keywords: Delayed life history effect, Winter phenotype, Polymorphism, Torpor, Molting, Metabolism

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Background

The ability to respond to day length (photoperiodism) allows animals to change phenotype across the annual cycle. Response to shortening photoperiod consists of morphological, physiological and behavioral adjustments and results in development of winter phenotype. In winter, small mammals, which are mostly long-day breeders, regress gonads and cease reproduction [1–3], decrease body mass (m_b) [4–6], some molt to white fur [7–10], and heterothermic species use torpor [11, 12]. These adjustments allow for energy savings and are considered beneficial for winter survival, yet individuals insensitive to changes in day length (nonresponding individuals), or individuals presenting only some of winter traits (partial-responding individuals) also exist in many populations [7, 8, 13–20]. The diversity of winter phenotypes may result from complexity of physiological and molecular mechanisms underlying photoresponsiveness [21, 22]. Melatonin, the hormonal signal of day length, enters multiple molecular pathways which control molting, torpor expression or gonadal regression [23, 24]. Although these pathways are often interrelated, winter traits may be regulated independently [23, 25–27].

According to the theory of delayed life history effects, phenotype of an adult individual results from environmental conditions experienced at birth, and later during growth and maturation [28–30]. Thus, it may be also affected by the time of birth during the reproductive season [31–33]. Late- and early born individuals differ in time it takes to reach puberty [34, 35], strategy of winter survival [33, 35], and longevity [36, 37]. Individuals born later during the reproductive season are often smaller [32, 38], grow slower [33] and have a lower probability of winter survival [36] than individuals born earlier. One can argue that late-born individuals have less time to grow and gather energy reserves before winter [32, 38].

In the Boreal and Temperate Zones seasonally changing day length correlates with changes in ambient temperature and resource availability. Day length experienced during development influences responsiveness to short day in adult Siberian hamsters *Phodopus sungorus*, a long-day breeder, which is a model animal in the studies of seasonal adjustments in physiology [16, 17, 39]. A majority of hamsters born and/or weaned under photoperiod shorter than 15 h respond to short days [17, 33], contrary to those born or weaned under 16 h photoperiod or longer [14, 15, 33]. Because photoperiod is related to time of the year, it has been proposed that nonresponsiveness to short days results from being born early in the reproductive season and exposure to long days during first weeks of life [17]. Butler et al. [33, 40] used simulated natural photoperiod to demonstrate that the proportion of nonresponding Siberian hamsters was greater in cohorts born under lengthening photoperiod (early born cohorts) than in cohorts born when days were shortening (late-born cohorts). In all cohorts responders always predominated, but even among hamsters born late in the season over 10% of individuals did not respond to short days [33]. This suggests that factors other than day length may play a role in development of the nonresponding phenotype.

Between April and September Siberian hamsters may deliver up to 5 or 6 litters [41], therefore photoperiod experienced by offspring at birth and during early development may considerably differ. Since being born late in the season is intrinsically related to shorter exposure to long photoperiod, it is hard to disentangle the effect of photoperiod and litter order on adult winter phenotype. In many mammalian species, both long- and short-day breeders, litters differ in offspring quality [42–45]. Depending on the species, subsequent litters can be bigger [44–46] or smaller [43, 47] or not different from each other [48, 49].

We proposed that extrinsic factors, such as access to energy resources prior to winter, influence the strategy of winter survival in small long-day breeding mammals and hypothesized that being born late in the reproductive season increases probability of subsequent development of photoresponsiveness. We aimed to experimentally distinguish the effect of litter order from the effect of exposure to long photoperiod (LP) before winter by manipulating the duration of LP acclimation in consecutive litters (Fig. 1). We predicted that the proportion of individuals developing traits characteristic for an energy-conserving phenotype (white fur, torpor use, and low m_b) would be greater among individuals exposed to long photoperiod for a short time, irrespective of the litter in which they were born. As a model we used the Siberian hamster, photosensitive rodent that exhibits a high level of polymorphism of winter phenotype, from responding individuals, through individuals which develop only some of winter traits, to nonresponding ones [13, 50, 51]. Additionally, we compared basal metabolic rate (BMR) of animals from different experimental groups to assess effect of litter order, age, photoperiod and winter phenotype traits on basal energy consumption.

Results

Effect of litter order and duration of LP acclimation on offspring body mass

Offspring m_b increased with dam m_b (LME: F (1, 23) = 5.951, $P = 0.026$) and was negatively correlated with litter size, but the latter was true only in first litters (litter order × litter size, LME: F(1, 372) = 5.978, $P = 0.015$). Offspring had similar m_b for the first nine days of life, irrespective of litter order, but thereafter offspring from first litters were smaller than third litter individuals (litter order × age, LME: F(1, 351) = 5.861, $P < 0.001$; Fig. 2). Although differences in body mass

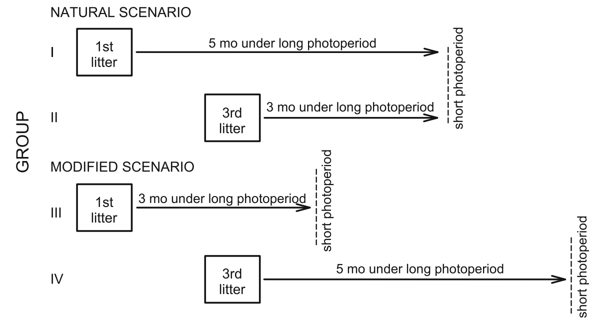

Fig. 1 Experimental design. Siberian hamsters were divided into four groups differing in time of birth (1st or 3rd litters) and duration of acclimation to long photoperiod (3 or 5 months). See Methods for details

between consecutive litters ranged from 9.56% at day 12 of life to 16.43% at day 15, it vanished between day 45 and day 90. However 60-day old hamsters from third litters were bigger than individuals from first litters (litter order × age, LME: F(1, 600) = 4.785, *P* = 0.003, Fig. 2). Between days 45 and 90 males from first and third litters did not differ but females born in first litter were smaller than those born in third litter (litter order × sex, LME: F(1, 485) = 6.581, *P* = 0.011). Body mass of offspring acclimated to LP for 3 or 5 months did not differ prior to transfer to SP (GLM: F(1, 199) = 1.608, *P* = 0.206). Model designs and results of the analysis of variance are given in Table 1, whereas data on hamster m_b are given in Table 2.

Parental effect on offspring phenotype

None of the parental pairs delivered offspring which developed only one phenotype (the same set of winter traits) in response to short photoperiod. In some pairs, most offspring turned white and used torpor, whereas in other pairs grey offspring that did not use torpor predominated. Different phenotypes among littermates were more common in first (in 24 out of 25 parental pairs) than in third litters (in 17 out of 25 parental pairs) (χ^2(1, 50) = 6.640, *P* = 0.010). For example, in three parental pairs littermates showed all possible combinations of winter traits, and therefore phenotypes: white fur and torpor use, white fur without torpor use, grey fur and torpor use, and grey fur without torpor use. In another 14 pairs, offspring presented three different phenotypes and in 8 pairs only two different combinations of traits. Because we did not know parental phenotype, we were not able to calculate heritability of winter traits. However, the goodness of fit of models with and without random effect of parental ID differed significantly (*P* = 0.001), suggesting a strong parental effect on offspring phenotype.

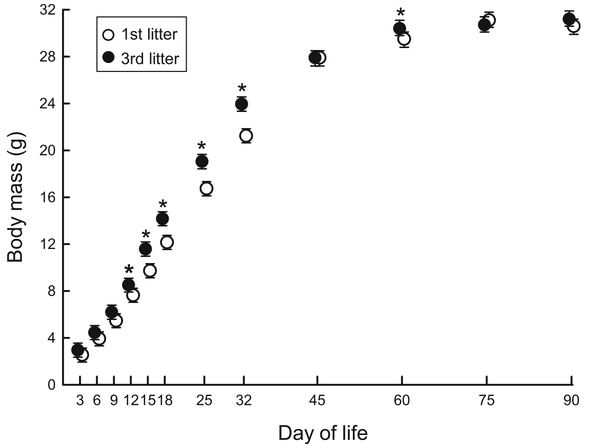

Fig. 2 Body mass of offspring born in 1st (white symbols) and 3rd (black symbols) litters. Dots indicate estimated marginal means, and whiskers are 95% confidence intervals. Asterisks show days when litters differed significantly

Effect of litter order and duration of LP acclimation on photoresponsiveness

During the first 16 weeks of acclimation to SP, 94 out of 200 animals used daily torpor and 125 animals molted to white fur. Between weeks 20 and 37 of acclimation to SP, another 23 animals molted and 29 entered torpor for the first time. Generally, experimental groups did not differ in propensity to use torpor (litter order × duration of LP acclimation χ^2 (1, 200) < 0.001, *P* = 0.993) or molting (litter order × duration of LP acclimation χ^2(1, 200) < 0.001, *P* = 0.988). Neither litter order (χ^2(2, 200) = 0.194, *P* = 0.907) nor duration of LP acclimation (χ^2(2, 200) = 1.660, *P* = 0.436; Table 3) affected the use of torpor. There was also no effect of litter order on molting (χ^2(2, 200) = 0.128, *P* = 0.938; Table 3). However, in groups acclimated to LP for 3 months we observed 71% individuals which molted to white in response to SP, and only 54% in groups acclimated to LP for 5 months (χ^2(2, 200) = 6.36, *P* = 0.041; Table 3). In the latter groups, we observed also almost two times more late-responding individuals than in groups acclimated to LP for 3 months (Table 3).

The higher the initial m_b of hamsters at the end of acclimation to long days, the greater the decrease of m_b after acclimation to short days (GLM: F(1, 198) = 14.82, *P* < 0.001). Neither litter order (GLM: F(1, 186) = 1.287, *P* = 0.258) nor duration of LP acclimation (GLM: F(1, 186) = 1.345, *P* = 0.248) affected m_b changes after 16 weeks in SP. We found that m_b change correlated with other winter phenotype traits. Namely, individuals using torpor (regardless of the time spent in SP) lost between 1 and 9% of initial m_b while individuals that did not use torpor maintained initial m_b or even gained it to 3% (GLM: F(1, 186) = 11.134, *P* < 0.001). Changes of m_b in

Table 1 Results of the type III analysis of variance calculated for offspring body mass in Siberian hamster

Model no	Trait	Model	Factors	F(df)	*P-value*
1	m_{b} until 32-day of life	LME	**age**	**633.54 (7, 55)**	**0.006**
			litter order	3.49 (1, 374)	0.062
			litter size	**9.45 (2, 283)**	**0.002**
			dam m_{b}	**5.95 (1, 23)**	**0.026**
			age × litter order	**5.86 (7, 351)**	**< 0.001**
			litter order×litter size	**5.98 (1, 372)**	**0.015**
2	m_{b} between 45 and 90-day of life	LME	**age**	**108.05 (3, 601)**	**< 0.001**
			litter order	0.58 (1, 188)	0.447
			sex	**318.83 (1, 485)**	**< 0.001**
			age × litter order	**4.78 (3, 601)**	**0.003**
			litter order × sex	**3.58 (1, 485)**	**0.011**
3	m_{b} prior to transfer to SP	GLM	litter order	2.44 (1, 199)	0.120
			duration of LP acclimation	1.61 (1,199)	0.206
			sex	**136.26 (1, 199)**	**<0.001**
4	m_{b} after 16 weeks under SP	GLM	litter order	0.81 (1, 186)	0.368
			duration of LP acclimation	1.55 (1, 186)	0.214
			sex	0.34 (1, 186)	0.563
			torpor use	**17.02 (2, 186)**	**< 0.001**
			molting	**81.71 (2, 186)**	**< 0.001**
			initial mb	**11.10 (1, 186)**	**0.001**
			sex × torpor use	**4.58 (2, 186)**	**0.034**
			sex × molt	**9.45 (2, 186)**	**0.002**

m_b body mass, *SP* short photoperiod, *LP* long photoperiod, *LME* linear mixed effect model, *GLM* general linear model. Significant effects are indicated in bold

different molting categories were related to sex (sex × molting GLM: F(1, 186) = 5.721, $P = 0.004$). Both, grey males and grey females gained up to 4.5% of initial m_{b}, whereas white males and white females lost between 9 and 14% of initial m_{b}. Within animals that molted after 20 weeks under SP, males gained around 5%, and females lost over 5% of initial m_{b}.

Changes of m_{b} during further acclimation to SP in late responding animals correlated with their phenotype. Animals that molted within 16 weeks in SP and started to use torpor later than 20 weeks in SP did not change their m_{b} any further. Most individuals that both molted and started to use torpor later than after 20 weeks in SP decreased their m_{b} by approximately 16.3 ± 10.2% (Fig. 3) but some of them also gained m_{b} by 10.1 ± 7.6%. The two grey individuals that started to use torpor later than after 20 weeks in SP differed between each other, one lost m_{b} while the other maintained constant m_{b}.

Basal metabolic rate

Basal metabolic rate was repeatable in 3-month-old animals ($\tau = 0.263$, $P = 0.001$) and between acclimation to long photoperiod and short photoperiod ($\tau = 0.235$, $P = 0.001$), but not in late responders between subsequent measurements ($\tau = 0.060$, $P = 0.151$).

Hamsters born in first or third litters did not differ in BMR at the age of 3 months (LMM: F(1, 217) = 1.486, $P = 0.224$) but after adjusting for m_{b} females had higher BMR than males (0.273 ± 0.002 W and 0.261 ± 0.002 W respectively; LMM: F(1.224) = 15.218, $P < 0.001$). Basal metabolic rate decreased from 0.261 ± 0.004 W in long photoperiod to 0.247 ± 0.004 W after 16 weeks of acclimation to short photoperiod (LMM: F(1.591) = 66.739, $P < 0.001$, Fig. 4). This decrease depended on molting category, litter order and duration of LP acclimation. Namely, individuals acclimated to long days for 3 months did not change BMR between photoperiods, whereas those acclimated to LP for 5 months decreased BMR after being transferred to short days (photoperiod × duration of LP acclimation LMM: F(1, 595) = 44.234, $P < 0.001$). While all individuals had similar BMR in long photoperiod, after 16 weeks under short photoperiod BMR did not differ only among hamsters from third litters. Among hamsters from first litters, grey individuals had 6–10% higher BMR than white ones, and also than individuals that molted to white fur later (photoperiod × litter order × molting; LMM: F(1, 590) =

Table 2 Estimated marginal means ± SE for body mass of offspring from first and third litters, calculated from models one and two (Table 1.), compared pairwise with Tukey's HSD test adjusted for multiple comparisons. Significant differences are indicated in bold

Day of life	**Body mass (g)**		**Post-hoc *P -value***
	1st litter	**3rd litter**	
3	2.54 ± 0.30	2.95 ± 0.30	0.274
6	3.91 ± 0.30	4.45 ± 0.30	0.159
9	5.46 ± 0.30	6.19 ± 0.30	0.055
12	7.63 ± 0.30	8.50 ± 0.29	**0.021**
15	9.72 ± 0.30	11.57 ± 0.30	**0.001**
18	12.14 ± 0.30	14.16 ± 0.30	**0.001**
25	16.74 ± 0.31	19.05 ± 0.31	**0.001**
32	21.24 ± 0.30	23.95 ± 0.31	**0.001**
45	27.90 ± 0.33	27.90 ± 0.33	0.998
60	29.50 ± 0.33	30.40 ± 0.33	**0.042**
75	31.10 ± 0.33	30.70 ± 0.33	0.437
90	30.60 ± 0.33	31.20 ± 0.33	0.174

Significant differences are indicated in bold

5.376, $P = 0.005$, Fig. 4). In late responding first litter individuals BMR increased in the course of acclimation to short photoperiod, whereas in individuals from third litters BMR was the same between measurements in short photoperiod (litter order × time of measurement LMM: $F(1, 139) = 3.615$, $P = 0.029$). Model designs and results of the analysis of variance are given in Table 4.

Discussion

Early life events may profoundly affect adult phenotype [31–33]. We found that individuals that spent 3 months under long photoperiod were more prone to molt to a white fur than individuals from groups acclimated to long photoperiod for 5 months (Table 3). Additionally, white individuals had lower basal energy expenditure in short photoperiod than grey ones (Fig. 4). This supports our prediction that individuals exposed to long photoperiod for a short time, irrespective of the litter in which they were born, were more prone to develop an energy-conserving phenotype. However, our prediction that individuals born later during reproductive season have less time to gather energy reserves before winter was not supported because initial differences in body mass between hamsters originating from consecutive litters vanished before transfer to short photoperiod (Fig. 2). We also found that litter order or duration of LP acclimation had no effect on torpor use or m_b change.

Delayed life history effect and offspring body mass

In the present study, consecutive litters differed in growth rate and individuals born in third litters grew faster than individuals born in first litters (Fig. 2, Table 2). In many mammalian species, including Siberian hamsters, litter order or time of birth during reproductive season affect litter or offspring quality [42–44, 46, 47]. It is true both for long- and short-day breeders. Long-day breeders, which are mostly small mammals with short gestation periods, mate in spring, while short-day breeders, like large ungulates, mate in autumn and have long gestation period. Although these groups differ in gestation lengths, parturition takes place at the time of year with highest resource availability. Therefore, late birth during the reproductive season may affect quality of mammalian offspring. Offspring of short-day breeder, red deer *Cervus elaphus*, born late during reproductive season were smaller than early born ones [36]. In European rabbits *Oryctolagus cuniculus* offspring from subsequent litters [47] were smaller than offspring born in earlier litters, whereas in garden dormice *Eliomys quercinus* [52], Uinta ground squirrels *Spermophilus armatus* [46], or Siberian hamsters [44] this trend was opposite. Differences in m_b between consecutive litters might have resulted from the efficiency of parental care, dam's age, and body condition. In European rabbits previous reproductive activity might have had negative effect on

Table 3 Effect of litter order and duration of long photoperiod (LP) acclimation on torpor use and molting in Siberian hamsters born in first or third litters, and acclimated to LP for 3 or 5 months. Each group consisted of 50 individuals acclimated to the short day for 16 weeks. Bold text indicates statistically significant differences (*P* < 0.05) between groups acclimated to LP for 3 or 5 months

		Torpor use			**Molting**		
		Number of individuals			**Number of individuals**		
Duration of LP acclimation (months)	Litter	using torpor	not using torpor	using torpor after 20 weeks under SP	molting to white	remaining grey	molting to white after 20 weeks in SP
3	1	24	20	6	**34**	12	**4**
	3	27	17	6	**37**	9	**4**
5	1	22	20	8	**28**	15	**7**
	3	21	20	9	**26**	16	**8**

Each group consisted of 50 individuals acclimated to the short day for 16 weeks

Values indicated in bold show significant differences ($P < 0.05$) between groups acclimated to LP for 3 or 5 months

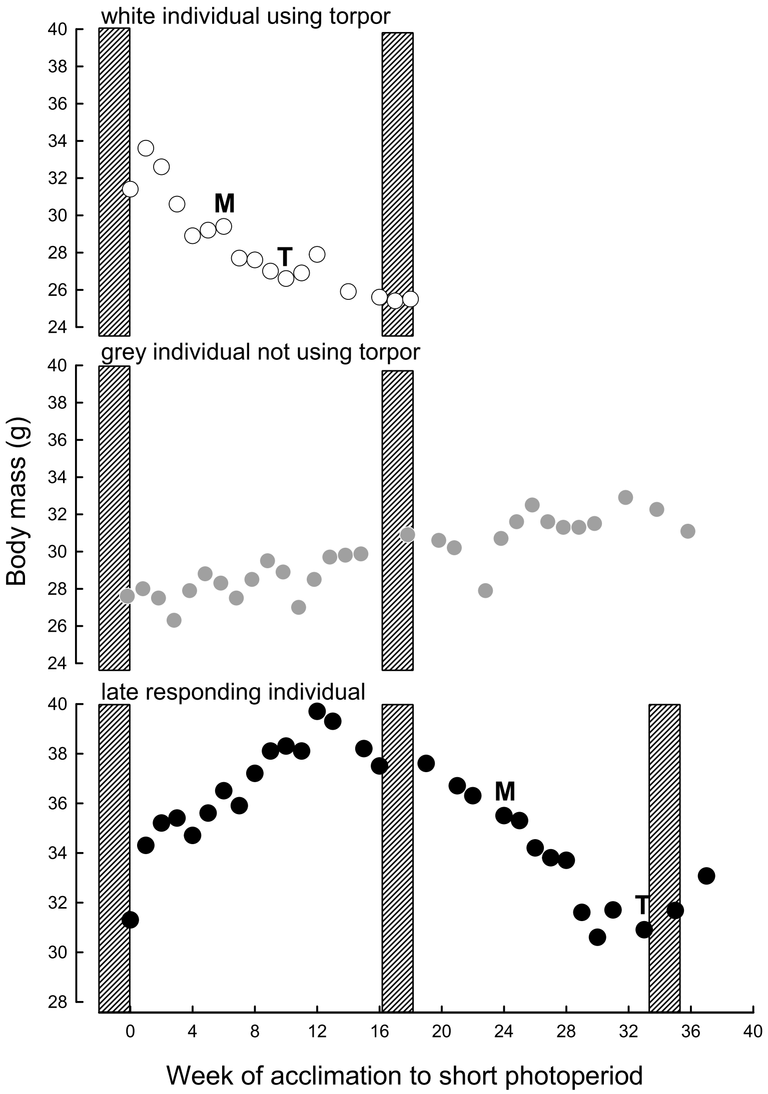

Fig. 3 Changes in body mass in representative white individual using torpor (top panel), grey individual not using torpor (middle panel) and individual using torpor and molt to white fur later than after 20 weeks under short photoperiod (SP) (bottom panel). White individuals using torpor were transferred to LP after BMR measurements in SP, and there are no data points afterward. Hatched bars indicate time of BMR measurements. M – stage 3 of molting; T – first episode of torpor

current reproduction since first litters had higher growth rate than consecutive ones [47]. Conversely, laboratory mice [53] and rats [54] increased lactation performance and therefore reproductive effectiveness with successive litters. However, increase in reproductive effectiveness was limited to first two or three litters, and then it constantly decreased [55, 56]. Quality of offspring might be related also to maternal age and body condition [55, 57]. Bigger dams of both long- and short-day breeders delivered bigger offspring [58–60], but reproductive effectiveness depended also on time of the year when the dam was born [55].

In a previous study we found that offspring m_b in Siberian hamsters was related to parental phenotype [44] and nonresponding hamsters delivered bigger offspring than responding ones. Since responding individuals regress gonads, energy allocated into gonadal recrudescence and rebuilding of body reserves may reduce resources available for reproduction. Presumably this is why photoresponding hamsters commenced breeding later than nonresponding ones [44]. Regardless of parental phenotype offspring from successive litters were always bigger than earlier ones ([44], present study). In the present study, a negative relationship between litter size and offspring m_b only in first litters may suggest that after winter all individuals, regardless of their photoresponsiveness and gonadal status, might be in negative energy balance. It clearly indicates that

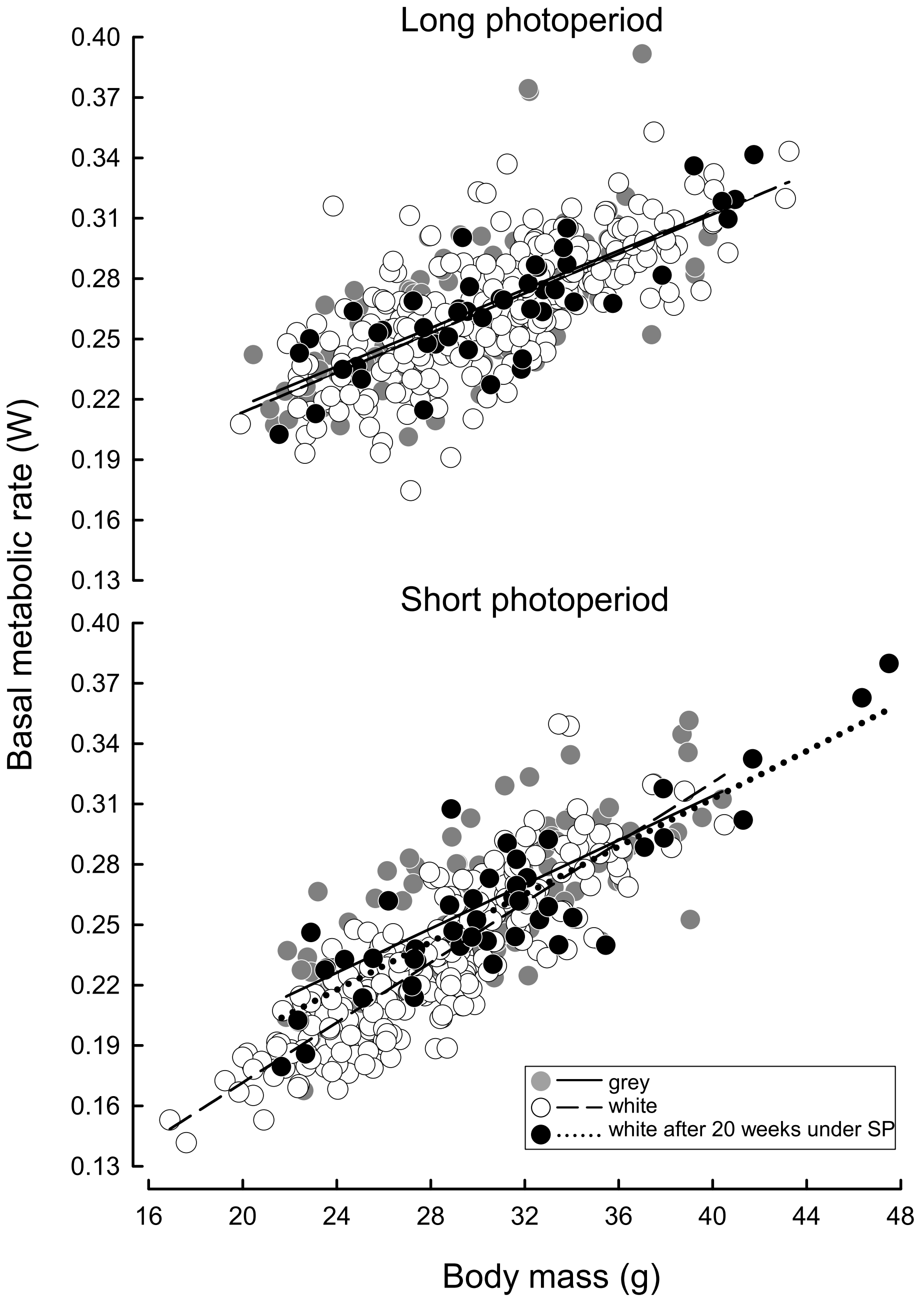

Fig. 4 Relationship between basal metabolic rate (BMR) and body mass in white and grey individuals and in individuals that molted to white after 20 weeks under short photoperiod. Top panel shows data recorded in long photoperiod prior transfer to short photoperiod, and bottom panel shows data recorded after 16 weeks in short photoperiod. Regression lines did not differ between groups in long photoperiod but did differ in short photoperiod (see text). BMR was measured at ~28.5 °C

reproduction-related trade-offs appear only when energy resources are limited.

We predicted that individuals born later in the reproductive season would develop traits of an energy-conserving phenotype, because these individuals have less time for gathering energy reserves, and they are considered to have lower chance to survive and reproduce next year [34, 38, 61]. On the one hand faster growth rate of offspring from subsequent litters could have compensated for late birth [46, 62, 63]. On the other hand, compensatory growth might have delayed consequences, such as inhibited growth in adults [64–66]. Since differences in m_b between litters vanished already at day 45 of life (Fig. 2), the latter explanation may be the case in Siberian hamsters. Under natural conditions, this species breeds from April to September [41]. Even if dams give birth every 23–

Table 4 Results of the type III analysis of variance calculated for basal metabolic rate (BMR) in Siberian hamster

Model no	Trait	Model	Factors	F(df)	*P-value*
1	BMR in 3 months old animals	LME	**m_b**	**189.79 (1, 222)**	**<0.001**
			litter order	1.49 (1, 208)	0.224
			sex	**15.22 (1, 224)**	**<0.001**
			m_b×litter order	2.87 (1, 209)	0.091
2	BMR after16 weeksunder SP	LME	**m_b**	**715.89 (1, 412)**	**< 0.001**
			litter order	0.10 (1, 191)	0.752
			duration of LP acclimation	0.37 (1, 190)	0.542
			sex	**24.43 (1, 274)**	**< 0.001**
			photoperiod	**66.74 (1, 591)**	**< 0.001**
			molting	**9.07 (2, 190)**	**0.001**
			duration of LP acclimation × molting	**3.31 (2, 189)**	**0.038**
			duration of LP acclimation × photoperiod	0.18 (1, 595)	0.673
			litter order × molting	**4.61 (2, 190)**	**0.011**
			litter order × photoperiod	2.92 (1, 590)	0.087
			photoperiod × molting	1.38 (2, 594)	0.252
			duration of LP acclimation × photoperiod × molting	0.35 (2, 591)	0.707
			litter order × photoperiod×molting	**5.38 (2, 590)**	**0.005**
3	BMR of late responding individuals	LME	**m_b**	**204.38 (1, 40)**	**<0.001**
			litter order	0.02 (1, 99)	0.877
			duration of LP acclimation	2.78 (1, 22)	0.109
			sex	**7.67 (1, 24)**	**0.011**
			time of measurement	**6.23 (2, 137)**	**0.002**
			molting	0.01 (1, 22)	0.940
			litter order × time of measurement	**3.62 (2, 139)**	**0.029**

m_b body mass, *SP* short photoperiod, *LP* long photoperiod, *LME* linear mixed effect model. Significant effects are indicated in bold

30 days [67], third litters are born in June or July, i.e. 2–3 months before winter. Since we did not find differences between litters in body mass or BMR at the age of 3 months and prior to transfer to SP, it suggests that this time is long enough to complete growth before winter.

Delayed life history effect and photoresponsiveness

We observed the whole spectrum of possible winter phenotypes among the study animals, including late responders that developed winter traits after more than 20 weeks under SP. It was previously reported that photoresponsiveness diminished with animal age and vanished after first year of life [6, 8]. However, since hamsters from first and third litters were born ~ 60 days apart, age cannot explain differences in photoresponsiveness.

Being born late in reproductive season decreased probability of winter survival in hibernating rodents [46, 68]. To our knowledge, direct effects of the duration of exposure to long photoperiod on physiology of seasonal rodents originating from early and late litters have not been tested before. Butler et al. [33, 40] found that more nonresponding hamsters were born in lengthening photoperiod (early cohorts), but nonresponding individuals were still present among individuals born under shortening photoperiod (late cohorts) [33, 40]. Nevertheless, the study did not report whether hamsters originated from first or consecutive litters.

Prolonged acclimation to LP reduced the number of individuals that molted to white fur in response to SP (Table 3). Seasonal molting is related to decreased prolactin level [21, 22]. Since Siberian hamsters born early during reproductive season may reach puberty before their first winter, prolonged acclimation to long photoperiod may increase prolactin level and therefore inhibit molting. Lack of molting can be regarded as disadvantageous because white fur increases insulation [69], but Boratyński et al. [51] did not find differences in thermal conductance between winter and summer acclimated Siberian hamsters. Although energy savings due to molting in small mammals are considered to be limited [9, 70], they may still be present. Here we found that after acclimation to short photoperiod, BMR of white animals was 10% lower than BMR of grey individuals (Fig. 4). Since high prolactin level has been shown to increase food intake, adipogenesis, and m_b [71, 72] it may also explain high BMR of grey individuals acclimated to short days.

Neither litter order nor duration of LP acclimation affected torpor use or m_b change. As far as we know, this is first study which experimentally tested the effect of these factors on torpor use in Siberian hamsters. Torpor is an effective way to reduce energy expenditure under SP [73–75] and propensity for torpor increased in cold or after fasting [76, 77]. It is plausible that we did not observe any effect of litter order and duration of LP acclimation on torpor use because of constant and relatively high ambient temperature during acclimation and food available ad libitum. This might also be a reason for similar m_b prior to transferring animals to SP and for similar time courses of m_b changes under SP in all groups. Although, individual body condition and energy reserves did not affect photoresponsiveness under mild conditions, it may be of great importance in harsh environments.

In the present study, prolonged acclimation to long days increased the number of late responding hamsters (Table 3). In these individuals the sequence of seasonal changes was the same as in typically responding ones (response after 12–16 weeks under SP). Namely, decrease in m_b was followed by molting and finally, occurrence of torpor (Fig. 3). However, late responders were in their winter phenotype for shorter time because photorefractoriness occurred at a similar time as during the typical response to SP [11, 78]. Although the phenomenon of late responders is known [11, 78], the mechanism of delayed response to winter remains unexplained. It does not seem to be a laboratory artefact, because both abrupt and gradual changes from long to short days led to development of winter phenotypes and any differences vanished before the 12th week of SP acclimation [16, 79].

At first glance, existence of late responders under natural conditions seems to be difficult to understand. However, this picture may change when we account for the fact that other winter-related phenomena, such as snowfall or low ambient temperature, may be shifted towards spring. Since late responders from third litters were able to maintain low BMR for the entire course of acclimation to short photoperiod, their energy reserves might have been maintained for a longer time. Therefore, prolonged cold or unexpected snowfall in spring may favor a late winter response and explain maintenance of late responders in a population.

Polymorphism in winter traits within a litter

In the present study none of the parental pairs delivered offspring of one phenotype. Within a litter there were individuals of different responsiveness to SP, but in a few parental pairs one offspring phenotype predominated, suggesting heritability of the winter phenotype. Previous reports showed that phenotype was heritable in Siberian hamsters and white-footed mice [3, 14, 80] and artificial selection led to increase of photoresponsiveness [81]. Since our colony is outbred, parental pairs were paired randomly and we did not control for their winter phenotype, calculation of heritability of winter traits was impossible. Polymorphism was more common in the first litters suggesting that individuals born earlier during the reproductive season may show higher variability in the response to shortening days, than less flexible, bigger and faster growing individuals from third litters. In our breeding colony some littermates that were maintained together in the same cage and under the same conditions developed different winter phenotypes (Fig. 5). According to Balanced Polyphenism Hypothesis [82, 83] offspring of different phenotypes may increase parental fitness, because each phenotype can be advantageous under specific environment conditions.

Conclusions

Winter phenotype may be considered as a suite of morphological, physiological and behavioral adjustments, but it seems that particular winter traits develop independently. To the best of our knowledge this is the first time when existence of different phenotypes among littermates is reported. Goldman and Goldman [15] proposed that all hamsters are born as responsive to short photoperiod but in some individuals this response is lost due

Fig. 5 Littermates of Siberian hamsters presenting different winter phenotypes. Left one remained grey and never used daily torpor, whereas right one molted to white and used daily torpor. Picture was taken after 14 weeks under short photoperiod (8L:16D)

to extrinsic factors (e.g. long photoperiod during postnatal development). Indeed, time of birth during the season may affect strategy of winter survival since prolonged acclimation to long days inhibited seasonal molting and increased energy expenditure. Individuals which do not respond to SP and have higher BMR may have a lower chance to survive harsh winter than individuals which responded to SP with all physiological and morphological traits. Conversely, nonresponding individuals may develop a late response which may be favored under particular environmental condition. Although we did not find any delayed life history effects on torpor use or seasonal m_b changes, we suggest that under harsh environmental conditions and when resources are limited, or in younger individuals, energy supplies gathered before winter have significant effect on development of these winter traits and overall photoresponsiveness.

Methods

Animals

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decisions: 8/2018, 22/2018, 48/2018, 18/2019). We used 200 Siberian hamsters, 100 males and 100 females, originating from our outbred colony maintained at the Faculty of Biological and Veterinary Sciences at the Nicolaus Copernicus University in Toruń, Poland. Half of animals were born in the first litters and another half in the third litters. All individuals were born between late May and late August. Litters were weaned at day 18 and each litter shared one cage until day 32. After that, we randomly selected 2 males and 2 females from each litter. Siblings of each sex were maintained together until they were two-months old. Thereafter each hamster was assigned to experimental group and transferred to an individual cage in which it was maintained throughout entire experiment. Groups did not differ in body mass at the age of 2 months (UNIANOVA F(1, 200) = 1.126, $P = 0.340$). To estimate growth rate, whole litters were weighed every 3 days until weaning and then every week until day 32. Weighing was continued every two weeks until transfer to short photoperiod. After birth hamsters were maintained under long photoperiod (LP; 16 L:8D, lights on at 04:30 a.m.) at ambient temperature of 20 ± 2 °C. The 16 h day does not inhibit further response to short photoperiod [14, 17]. All animals were maintained in standard laboratory cages (Tecniplast, 1245, Italy, 33 × 20 × 18 cm) with deciduous wood chips as bedding material. Hamsters were fed with standard rodent food with higher content of protein and fat (60% of carbohydrates, 10% of fat and 30% of protein; Labofeed H standard, Morawski, Kcynia, Poland) until the second month of life, and afterwards with standard maintenance food (67% of carbohydrates, 8% of fat and 25% of protein; Labofeed B standard, Morawski, Kcynia, Poland). Animals were supplied with drinking water ad libitum.

Experimental protocol

To determine the effect of time of birth on offspring photoresponsiveness, we set four experimental groups of 50 individuals each, which differed in the time of birth or in the duration of acclimation to long photoperiod after birth (Fig. 1). Although Siberian hamsters may breed immediately after parturition of previous litter [41, 84], a post-implantation embryonic diapause is common [67] and females usually deliver consecutive litters every ~ 23–30 days [44]. In our study third litters were ~ 2 months (50–65 days) younger than the first litters. Hamsters originating from first and third litters were randomly assigned to experimental groups which differed in duration of post-natal acclimation to long photoperiod. Two groups were intended to imitate natural conditions (Natural Scenario in Fig. 1). Group I was composed of animals born in first litters that were acclimated to LP for ~ 5 months and then transferred to short photoperiod (SP; 8 L:16D, lights on at 08:30 a.m.). Hamsters from Group II were born in third litters and since they were transferred to SP together with the first group, they were acclimated to LP for ~ 3 months. Groups III and IV were used to control for the effects of litter order and duration of LP acclimation on the development of winter traits (Modified Scenario in Fig. 1). Individuals from group III were born in the first litters, but their acclimation to LP was shortened to 3 months. In group IV, LP acclimation of animals born in the third litters was lengthened to ~ 5 months. To test our hypothesis, we used abrupt change of photoperiod, which allowed to precisely measure time spent in both photoperiodic regimes. Despite limitations discussed by Gorman et al. [16], this protocol has been widely used to induce a seasonal response in Siberian hamsters [7, 14, 85–89]. It has also been reported that proportion of nonresponding individuals was not related to the method of photoperiod transition from summer to winter [16]. To account for parental effect, all offspring were derived from 25 parental pairs. Each parental pair was represented by one male and one female offspring in each group, resulting in 8 offspring per parental pair across all groups.

Determination of winter traits

After transfer to SP, hamsters were maintained individually, in the same type of cages and at the same ambient temperature as in LP. Development of winter traits was determined after 16 weeks under SP based on pelage color, torpor use and m_b change. Traditionally, individuals were classified as responders, nonresponders, or partial responders based on set of several winter traits, such as daily torpor and pelage color or gonadal regression and m_b loss [8, 13, 40, 90]. However, because winter traits are controlled by different hormonal and molecular pathways [21, 22] and they may develop independently in response to SP, such simple classifications become debatable. Here, we analyzed effect of delayed history effects on each winter trait separately. An individual was classified as using torpor if it entered at least one torpor episode (subcutaneous temperature ≤ 32 °C, stereotypical posture, reduced responsiveness). To determine this, hamsters were injected subcutaneously in interscapular region with thermosensitive passive integrated transponders (BioTherm 13, Biomark, Boise, ID, USA) after 2 to 4 weeks of SP acclimation. Although the manufacturer-reported temperature range of transponders is 33 °C to 43 °C, we calibrated them in a water bath against a high-precision mercury-in-glass thermometer between 27.0 °C and 40.0 °C, which allowed us to measure subcutaneous temperature (T_{sc}) lower than 30 °C. Any T_{sc} lower than 24 °C was indicated on the reader as "Low". These transponders allowed for remote monitoring of T_{sc} and torpor use during acclimation. Daily controls of hamster T_{sc} began four weeks after implantation. Between 10:30 and 14:30 (2 to 4 h after lights on), at a random time, we read T_{sc} with a remote reader (HPR plus, Biomark, Boise, ID, USA) and additionally noted if animal were in characteristic torpid ball-shape posture and whether they responded to gentle opening of the cage. We classified individuals as white, if we observed at least stage 3 of winter fur according to Figala scale (where 1 is dark grey with black stripe on a back and 6 is white without a stripe [91]). We did not score the intensity of fur color change any further. To assess m_b changes under SP, hamsters were weighed (to the nearest 0.1 g; Scout Pro 200, Ohaus, USA) every week during first 16 weeks of acclimation to SP and then every one or two weeks until week 40. In some individuals, so-called "late responders", winter traits may appear even after 32–37 weeks under SP [11]. Hence, all hamsters that did not develop either white fur or torpor use after 16 weeks were maintained under SP up to ~ 40 weeks.

Measurement of basal metabolic rate

Basal metabolic rate was measured in normothermic hamsters at the age of three months. In groups I and IV (Fig. 1), which were acclimated to LP for 5 months, BMR was additionally measured at the age of five months, just before transfer to SP. Then, BMR was measured in all animals again after 16 weeks under SP. In late responders BMR was measured only if we observed torpor use for the first time after 20 weeks in SP and this measurement was done as soon as torpor was observed. Measurements were always done in a repetition, 7 days apart, to assess repeatability of BMR. Animals were weighed before and after each metabolic measurement.

Basal metabolic rate was measured by indirect calorimetry using an open-flow respirometry system (Sable Systems International, Las Vegas NV, USA; henceforth: SSI), as described in Jefimow et al. [92]. Measurements were done in the thermoneutral zone of Siberian hamster (T_a ~ 28.5 °C; [93]) and gas exchange was measured for ~ 7 h which is long enough to ensure post-absorptive state in this species [93]. BMR was calculated as a rate of the most stable 2 min of O_2 consumption during last three hours of the test. Animals were sealed in 0.85 L chambers made of polypropylene food containers (HPL 808, Lock&Lock, Hana Cobi, South Korea) which were placed in a temperature-controlled cabinet (ST-1200 BASIC, Pol-Eko-Aparatura, Wodzisław Śląski, Poland). We measured respiratory gas exchange of 14 animals simultaneously, using two parallel respirometry systems. Air was pulled from outside the building using an air pump (DOA-P501-BN, Gast Manufacturing INC., Michigan, USA) and dried in columns of silica gel. The main air stream was split into chambers and a reference gas stream. We regulated flow rate at ~ 500 ml min^{-1} upstream of each respirometry chamber using a precise needle valve. After air left chambers, a computer-controlled multiplexer (Intelligent Multiplexer V3, SSI) sequentially selected gases from each chamber. Gas exchange of each animal was measured for 5 min every 44 min with reference gas concentration readings every 20 min. Air flow was measured downstream of animal chambers using mass flow meter (FlowBar-8, SSI). Then the air stream was subsampled at ~200 ml min^{-1} and water vapor pressure of the subsampled air was measured with a water vapor analyzer (RH-300, SSI). Air was then dried using a nafion dryer tube (product number 17049, VacuMed, Ventura, CA, USA) embedded in silica gel, and a column of magnesium perchlorate (product number 11636.36, VWR International, Gdańsk, Poland). Subsequently, concentrations of CO_2 and O_2 were measured. In one system, we measured both rates of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$). In the second system, only $\dot{V}O_2$ was measured. We used O_2 (FC-10a, SSI) and CO_2 (CA-10, SSI) analyzers. All elements of the respirometry system were connected to PC via an analog-to-digital interface (UI2, SSI). Data were acquired using ExpeData software (SSI) at 0.5 Hz.

Metabolic rate (MR, *W*) was calculated using oxyjoule equivalent calculated after Lighton et al. [94]:

$$\mathrm{MR}(W) = \frac{\dot{V}O_2(16 + 5.164 \times RER)}{60},$$

where $\dot{V}O_2$ was the oxygen consumption (ml O_2 min^{-1}) and $RER = \frac{\dot{V}\,CO_2}{\dot{V}\,O_2}$. Because we did not measure $\dot{V}CO_2$ in one of the systems, we used RER = 0.80 which leads to smallest error in the calculation of energy expenditure [95].

Statistical analysis

Statistical modeling was done in R using packages lme4 [96] and stats [97], and package emmeans [98] for post-hoc comparisons of estimated marginal means. Initial maximal models were built using ecologically relevant explanatory variables and their interactions, which follows the Regression with Empirical Variable Selection approach [99]. We also used second-order Akaike information criteria (AICc) for post-hoc model comparisons (MuMIn [100]). All results are presented as estimated marginal means ± SE compared pairwise with Tukey's HSD test adjusted for multiple comparisons [98]. Statistical significance was accepted at $P \leq 0.050$.

First, we compared life history traits of individuals from all experimental groups. Up to 32 day of life m_b of individuals was determined as the mass of whole litter divided by litter size. Between 45 and 90 days of life all hamsters were weighed approximately once a week (± three days). Analysis of life history traits was done in three steps: 1) analysis of m_b measured before separation of siblings (growth rate between birth and day 32); 2) analysis of m_b between days 45 and 90; 3) analysis of m_b just prior to transfer to SP. The first two analyses were done using linear mixed-effect modeling (LME; lme4 [96]) with type III Sums of Squares. The last analysis was done using general linear model (GLM; stats [97]).

In the model which tested the effect of litter order on offspring m_b up to 32 day of life, parental ID was included as a random factor to control for repeated measurements. The final model included litter order (first or third litter), offspring age, litter size, m_b of dam, and litter order × age and litter order × litter size interactions (Table 1). In analysis of offspring m_b between ~ 45 and ~ 90 day of life, animal ID was used as a random factor, and litter order, sex and age were used as fixed factors. The best model also included interactions of age × litter order and litter order × sex (Table 1). In the analysis that compared m_b of individuals acclimated to LP for 3 (groups II and III) and 5 months (groups I and IV) prior to transfer to SP, litter order, duration of LD acclimation, and sex were included as fixed factors and the final model included only main effects of these variables (Table 1).

To test the effect of litter order and duration of LP acclimation on offspring photoresponsiveness we used contingency tables with Pearson's χ^2 test (stats [97]). To do so we defined categories of torpor use and molting. Molting categories included white individuals if they molted after 16 weeks under SP or grey if they did not molt at all. A separate category included individuals that presented late response and molted later than after 20 weeks under SP. Torpor categories comprised individuals using or not using torpor within 16 weeks of acclimation to SP and individuals using torpor later than after 20 weeks in SP. Effects of litter order and duration of LP acclimation were tested separately. We tested also for the effect of interaction of the above factors on molting and torpor use using Cochran-Mantel-Haenszel test (stats [97]).

To assess the effect of litter order and duration of LP acclimation on m_b changes triggered by SP we used GLM (stats [97]). We calculated proportional change of m_b using last measurement taken in LP as the initial m_b. Litter order, duration of LP acclimation, sex, categories of torpor use and molting were included as fixed factors and initial m_b as a covariate. The final model included all factors and interactions of sex × torpor use and sex × molting (Table 1).

Changes of m_b between 16th and 40th week of acclimation to SP in late responding individuals are presented descriptively because these individuals differed in winter traits and also in timing of their development.

To test weather polymorphism of winter phenotype among littermates was affected by litter order we used contingency tables with Pearson's χ^2 test. We classified litters as homogenous if all littermates presented the same phenotype and heterogeneous if littermates presented varied phenotypes.

To test for the effect of litter order and time spent in LP on BMR we used LME (lme4 [96]). We did three separate analyses: 1) to test for the effect of litter order on BMR in 3-months-old animals, 2) to test for the effect of litter order and duration of LP acclimation on changes in BMR triggered by photoperiod, and 3) to analyze changes in BMR in late responding individuals. All models included animal ID and trial as random factors that allowed to control for repeated measurements. Body mass was always included as covariate. The analysis of BMR in 3-months old animals included litter order and sex as fixed factors and their interaction (Table 4). To test for the effect of experimental group and development of winter traits on photoperiod-triggered changes in BMR we used litter order, duration of LP acclimation, photoperiod, sex, and winter traits (molting and torpor use) as fixed factors. The final model included fixed factors except torpor use and all interactions between duration of LP acclimation, photoperiod and molting and between litter order, photoperiod and molting (Table 4). In analysis of further changes of BMR in late responding individuals litter order, duration of LP acclimation, sex, molting and time of measurement (in LP, in SP and later in SP) were included as fixed factors. The final model included all main effects and interaction of litter order and time of measurement (Table 4).

Abbreviations

BMR: Basal metabolic rate; LP: Long photoperiod; m_b: Body mass; SP: Short photoperiod

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Consent for participate

Not applicable.

Authors' contributions

ASPP- concept of study, data acquisition, analysis and interpretation of data, writing the first draft of the manuscript and revisions. MJ - data acquisition, revision of the manuscript. MSW – help with data analysis, revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Code will be available upon request to the authors.

Declarations

Ethics approval and consent to particpate

All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decisions: 8/2018, 22/2018, 48/2018, 18/2019).

Consent for publication

All authors agreed to be listed and approved the manuscript and this submission.

Competing interests

The authors declare that they have no conflict of interest.

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References

1. Broussard DR, Robertson JL, Evans TJ, Faucher GK, Semanik MG, Heideman PD. Age-related decline in reproductive sensitivity to inhibition by short photoperiod in Peromyscus Leucopus. J Mammal. 2009;90(1):32–9.
2. Kaseloo PA, Crowell MG, Jones JJ, Heideman PD. Variation in basal metabolic rate and activity in relation to reproductive condition and photoperiod in white-footed mice (Peromyscus leucopus). Can J Zool. 2012; 90(5):602–15.
3. Kaseloo PA, Crowell MG, Heideman PD. Heritable variation in reaction norms of metabolism and activity across temperatures in a wild-derived population of white-footed mice (Peromyscus leucopus). J Comp Physiol B. 2014;184(4):525–34.
4. Hoffmann K. The influence of photoperiod and melatonin on testis size, body weight and pelage colour in the Djungarian hamster. J Comp Physiol. 1973;85:267–82.
5. Moffatt CA, Devries AC, Nelson RJ. Winter adaptations of male deer mice (Peromyscus-Maniculatus) and prairie voles (Microtus-Ochrogaster) that vary in reproductive responsiveness to photoperiod. J Biol Rhythm. 1993;8(3):221–32.
6. Bernard DJ, Losee-Olson S, Turek FW. Age-related changes in the photoperiodic response of Siberian hamsters. Biol Reprod. 1997;57(1):172–7.
7. Freeman DA, Goldman BD. Evidence that the circadian system mediates photoperiodic nonresponsiveness in Siberian hamsters: the effect of running wheel access on photoperiodic responsiveness. J Biol Rhythm. 1997;12(2):100–9.
8. Freeman DA, Goldman BD. Photoperiod nonresponsive Siberian hamsters: effect of age on the probability of nonresponsiveness. J Biol Rhythm. 1997; 12(2):110–21.
9. Hart JS. Seasonal changes in insulation of the fur. Can J Zool. 1965;34:53–7.
10. Kuhlmann MT, Clemen G, Schlatt S. Molting in the Djungarian hamster (Phodopus sungorus Pallas): seasonal or continuous process? J Exp Zool A Comp Exp Biol. 2003;295(2):160–71.
11. Lynch GR, Puchalski W. Effect of prolonged short day exposure on thermoregulation in the djungarian hamster (*Phodopus sungorus*). Living in the Cold: Physiological and Biochemical Adaptations. New York: Elsevier Sciensce Publishing; 1986.
12. Geiser F, Ruf T. Hibernation versus daily torpor in mammals and birds - physiological variables and classification of torpor patterns. Physiol Zool. 1995;68(6):935–66.
13. Przybylska AS, Wojciechowski MS, Jefimow M. Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behaviour. Anim Behav. 2019;147:105–14.
14. Goldman SL, Dhandapani K, Goldman BD. Genetic and environmental influences on short-day responsiveness in Siberian hamsters (Phodopus sungorus). J Biol Rhythm. 2000;15(5):417–28.
15. Goldman SL, Goldman BD. Early photoperiod history and short-day responsiveness in Siberian hamsters. J Exp Zool A Comp Exp Biol. 2003; 296(1):38–45.
16. Gorman MR, Freeman DA, Zucker I. Photoperiodism in hamsters: abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. J Biol Rhythm. 1997;12(2):122–35.
17. Gorman MR, Zucker I. Environmental induction of photononresponsiveness in the Siberian hamster, *Phodopus sungorus*. Am J Physiol. 1997;272(3 Pt 2): R887–95.
18. Zimova M, Mills LS, Nowak JJ. High fitness costs of climate change-induced camouflage mismatch. Ecol Lett. 2016;19(3):299–307.
19. Mills LS, Bragina EV, Kumar AV, Zimova M, Lafferty DJR, Feltner J, et al. Winter color polymorphisms identify global hot spots for evolutionary rescue from climate change. Science. 2018;359(6379):1033–6.
20. Atmeh K, Andruszkiewicz A, Zub K. Climate change is affecting mortality of weasels due to camouflage mismatch. Sci Rep. 2018;8(1):7648.
21. Hut RA, Dardente H, Riede SJ. Seasonal timing: how does a hibernator know when to stop hibernating? Curr Biol. 2014;24(13):R602–5.
22. Dardente H, Wood S, Ebling F, Saenz de Miera C. An integrative view of mammalian seasonal neuroendocrinology. J Neuroendocrinol. 2019;31(5): e12729.
23. Dardente H, Hazlerigg DG, Ebling FJ. Thyroid hormone and seasonal rhythmicity. Front Endocrinol (Lausanne). 2014;5:19.
24. Korf HW. Signaling pathways to and from the hypophysial pars tuberalis, an important center for the control of seasonal rhythms. Gen Comp Endocrinol. 2018;258:236–43.
25. Johnston JD, Ebling FJ, Hazlerigg DG. Photoperiod regulates multiple gene expression in the suprachiasmatic nuclei and pars tuberalis of the Siberian hamster (Phodopus sungorus). Eur J Neurosci. 2005;21(11):2967–74.
26. Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM, et al. Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. Endocrinology. 2012;153(1):101–12.
27. Ebling FJ. On the value of seasonal mammals for identifying mechanisms underlying the control of food intake and body weight. Horm Behav. 2014; 66(1):56–65.
28. Huck UW, Labov JB, Lisk RD. Food restricting young hamsters (Mesocricetus auratus) affects sex ration and growth of subsequent offspring. Biol Reprod. 1986;35:592–8.
29. Mitchell GW, Guglielmo CG, Wheelwright NT, Freeman-Gallant CR, Norris DR. Early life events carry over to influence pre-migratory condition in a free-living songbird. PLoS One. 2011;6(12):e28838.

30. Helle H, Koskela E, Mappes T. Life in varying environments: experimental evidence for delayed effects of juvenile environment on adult life history. J Anim Ecol. 2012;81(3):573–82.
31. Searcy CA, Gray LN, Trenham PC, Shaffer HB. Delayed life history effects, multilevel selection, and evolutionary trade-offs in the California tiger salamander. Ecology. 2014;95(1):68–77.
32. Rogovin KA, Bushuev AV, Khruscheva AM, Vasilieva NY. Resting metabolic rate, stress, testosterone, and induced immune response in spring- and fall-born males of Campbell's dwarf hamsters: maintenance in long-day conditions. Biol Bull Rev. 2014;4(3):181–91.
33. Butler MP, Trumbull JJ, Turner KW, Zucker I. Timing of puberty and synchronization of seasonal rhythms by simulated natural photoperiods in female Siberian hamsters. Am J Physiol Regul Integr Comp Physiol. 2007; 293(1):R413–20.
34. Ergon T, Lambin X, Stenseth NC. Life-history traits of voles in a fluctuating population repsond to immediate environment. Nature. 2001;411:1043–5.
35. Butler MP, Zucker I. Seasonal pelage changes are synchronized by simulated natural photoperiods in Siberian hamsters (Phodopus sungorus). J Exp Zool A Ecol Genet Physiol. 2009;311(7):475–82.
36. Clutton-Brock TH, Major M, Albon SD, Guinness FE. Early development and population dynamics in Red Deer. I. Density-dependent effects on juvenile survival. J Anim Ecol. 1987;56(1):53–67.
37. Rose KE, Clutton-Brock TH, Guinness FE. Cohort variation in male survival and lifetime breeding success in red deer. J Anim Ecol. 1998;67(6):979–86.
38. Plard F, Gaillard JM, Coulson T, Hewison AJM, Douhard M, Klein F, et al. The influence of birth date via body mass on individual fitness in a long-lived mammal. Ecology. 2015;96(6):1516–28.
39. Gorman MR, Zucker I. Seasonal adaptations of Siberian hamsters. II. Pattern of change in day length controls annual testicular and body weight rhythms. Biol Reprod. 1995;53:116–25.
40. Butler MP, Turner KW, Park JH, Butler JP, Trumbull JJ, Dunn SP, et al. Simulated natural day lengths synchronize seasonal rhythms of asynchronously born male Siberian hamsters. Am J Physiol Regul Integr Comp Physiol. 2007;293(1):R402–12.
41. Ross PD. Phodopus sungorus. Mamm Species. 1998;595:1–9.
42. Ingram DL, Mandl AM, Zuckerman S. The influence of age on litter-size. J Endocrinol. 1958;17(3):280–5.
43. O'Riain MJ, Jarvis JUM. The dynamics of growth in naked mole-rats: the effects of litter order and changes in social structure. J Zool. 1998;246:49–60.
44. Przybylska AS, Wojciechowski MS, Jefimow M. Photoresponsiveness affects life history traits but not oxidative status in a seasonal rodent. Front Zool. 2019;16(1):11.
45. Koketsu Y, Tani S, Iida R. Factors for improving reproductive performance of sows and herd productivity in commercial breeding herds. Porcine Health Manag. 2017;3:1.
46. Rieger JF. Body size, litter size, timing of reproduction, and juvenile survival in the Uinta ground squirrel, *Spermophilus armatus*. Oecologia. 1996;107(4): 463–8.
47. Rodel HG, Prager G, Stefanski V, von Holst D, Hudson R. Separating maternal and litter-size effects on early postnatal growth in two species of altricial small mammals. Physiol Behav. 2008;93(4–5):826–34.
48. Milligan BN, Fraser D, Kramer DL. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. Livest Prod Sci. 2002;76(1–2):181–91.
49. Myers P, Master LL. Reproduction by Peromyscus-Maniculatus - size and compromise. J Mammal. 1983;64(1):1–18.
50. Prendergast BJ, Kriegsfeld LJ, Nelson RJ. Photoperiodic polyphenisms in rodents: neuroendocrine mechanisms, costs, and functions. Q Rev Biol. 2001;76(3):293–325.
51. Boratyński JS, Jefimow M, Wojciechowski MS. Phenotypic flexibility of energetics in acclimated Siberian hamsters has a narrower scope in winter than in summer. J Comp Physiol B. 2016;186(3):387–402.
52. Mahlert B, Gerritsmann H, Stalder G, Ruf T, Zahariev A, Blanc S, et al. Implications of being born late in the active season for growth, fattening, torpor use, winter survival and fecundity. Elife. 2018;7:e31255.
53. Sadowska J, Gębczyński AK, Paszko K, Konarzewski M. Milk output and composition in mice divergently selected for basal metabolic rate. J Exp Biol. 2015;218(Pt 2):249–54.
54. Fischbeck KL, Rasmussen KM. Effect of repeated reproductive cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. J Nutr. 1987;117(11):1967–75.
55. Havelka MA, Millar JS. Maternal age drives seasonal variation in litter size of Peromyscus leucopus. J Mammal. 2004;85(5):940–7.
56. Fuchs S. Optimality of parental investment - the influence of nursing on reproductive success of mother and female young house mice. Behav Ecol Sociobiol. 1982;10(1):39–51.
57. Edwards HE, Tweedie CJ, Terranova PF, Lisk RD, Wynne-Edwards KE. Reproductive aging in the Djungarian hamster, *Phodopus campbelli*. Biol Reprod. 1998;58(3):842–8.
58. Lim JN, Senior AM, Nakagawa S. Heterogeneity in individual quality and reproductive trade-offs within species. Evolution. 2014;68(8):2306–18.
59. Cameron H, Monro K, Malerba M, Munch S, Marshall D. Why do larger mothers produce larger offspring? A test of classic theory. Ecology. 2016;97(12):3454–9.
60. Monaghan P. Early growth conditions, phenotypic development and environmental change. Philos Trans R Soc Lond Ser B Biol Sci. 2008; 363(1497):1635–45.
61. Prevot-Julliard AC, Henttonens H, Yoccoz NG, Stenseth NC. Delayed maturation in female bank voles: optimal decision or social constraint? J Anim Ecol. 1999;68:684–97.
62. Stumpfel S, Bieber C, Blanc S, Ruf T, Giroud S. Differences in growth rates and pre-hibernation body mass gain between early and late-born juvenile garden dormice. J Comp Physiol B. 2017;187(1):253–63.
63. Michel ES, Demarais S, Strickland BK, Wang G. Birth date promotes a tortoise or hare tactic for body mass development of a long-lived male ungulate. Oecologia. 2018;186(1):117–28.
64. Metcalfe NB, Monaghan P. Compensation for a bad start: grow now, pay later? Trends Ecol Evol. 2001;16(5):254–60.
65. Bertram DF, Chambers RC, Leggett WC. Negative correlations between larval and juvenile growth-rates in winter flounder - implications of compensatory growth for variation in size-at-age. Mar Ecol Prog Ser. 1993; 96(3):209–15.
66. Auer SK, Arendt JD, Chandramouli R, Reznick DN. Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (Poecilia reticulata). Ecol Lett. 2010;13(8):998–1007.
67. Newkirk KD, McMillan HJ, Wynne-Edwards KE. Length of delay to birth of a second litter in dwarf hamsters (Phodopus): evidence for post-implantation embryonic diapause. J Exp Zool. 1997;278(2):106–14.
68. Blumstein DT, Arnold W. Ecology and Social Behavior of Golden Marmots (*Marmota caudata aurea*). J Mammal. 1998;79(3):873–86.
69. Heldmaier G, Steinlechner S. Seasonal control of energy requirements for thermoregulation in the Djungarian hamster (Phodopus sungorus), living in natural photoperiod. J Comp Physiol. 1981;142(4):429–37.
70. Scholander PF, Hock R, Walters V, Irving L. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. Biol Bull. 1950;99(2):259–71.
71. Ben-Jonathan N, Hugo ER, Brandebourg TD, LaPensee CR. Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab. 2006;17(3):110–6.
72. Yahata T., Kuroshima A. Role of prolactin in brown adipose tissue thermogenic activity. In: Milton A.S. (eds) Temperature Regulation. Advances in Pharmacological Sciences. Basel: Birkhäuser; 1994.
73. Heldmaier G, Steinlechner S. Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*. Oecologia. 1981;48(2): 265–70.
74. Kirsch R, Ouarour A, Pevet P. Daily torpor in the Djungarian hamster (Phodopus sungorus): photoperiodic regulation, characteristics and circadian organization. J Comp Physiol A. 1991;168(1):121–8.
75. Ruf T, Heldmaier G. The impact of daily torpor on energy-requirements in the Djungarian hamster, Phodopus-Sungorus. Physiol Zool. 1992;65(5):994–1010.
76. Cubuk C, Bank JH, Herwig A. The chemistry of cold: mechanisms of torpor regulation in the Siberian hamster. Physiology (Bethesda). 2016;31(1):51–9.
77. Ruf T, Stieglitz A, Steinlechner S, Blank JL, Heldmaier G. Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (Phodopus sungorus). J Exp Zool. 1993;267(2):104–12.
78. Lynch GR, Wichman HA. Reproduction and thermoregulation in peromyscus: effects of chronic short days. Physiol Behav. 1981;26(2):201–5.
79. Hoffmann K, Illnerova H. Photoperiodic effects in the Djungarian hamster. Rate of testicular regression and extension of pineal melatonin pattern depend on the way of change from long to short photoperiods. Neuroendocrinology. 1986;43(3):317–21.
80. Heideman PD, Bronson FH. Characteristics of a genetic polymorphism for reproductive photoresponsiveness in the white-footed mouse (Peromyscus leucopus). Biol Reprod. 1991;44(6):1189–96.

81. Kliman RM, Lynch GR. Evidence for independence of circadian characters and extent of photoresponsiveness in the Djungarian hamster, *Phodopus sungorus*. J Biol Rhythms. 1991;6(2):159–66.
82. Nelson RJ. Photoperiod-nonresponsive morphs: a possible variable in microtine population-density fluctuations. Am Nat. 1987;130(3):350–69.
83. Ford EB. Polymorphism. Biol Rev Camb Philos Soc. 1945;20(2):73–88.
84. Wynne-Edwards KE. Evolution of parental care in Phodopus: conflict between adaptations for survival and adaptations for rapid reproduction. Am Zool. 1998;38(1):238–50.
85. Lynch EWJ, Coyle CS, Stevenson TJ. Photoperiodic and ovarian steroid regulation of histone deacetylase 1, 2, and 3 in Siberian hamster (Phodopus sungorus) reproductive tissues. Gen Comp Endocrinol. 2017;246:194–9.
86. Banks R, Delibegovic M, Stevenson TJ. Photoperiod- and Triiodothyronine-dependent regulation of reproductive neuropeptides, Proinflammatory cytokines, and peripheral physiology in Siberian hamsters (Phodopus sungorus). J Biol Rhythm. 2016;31(3):299–307.
87. Boratyński JS, Jefimow M, Wojciechowski MS. Individual differences in the phenotypic flexibility of basal metabolic rate in Siberian hamsters are consistent on short- and long-term timescales. Physiol Biochem Zool. 2017;90(2):139–52.
88. Wade GN, Bartness TJ. Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. Am J Phys. 1984;246(1 Pt 2):R26–30.
89. Bartness TJ, Clein MR. Effects of food deprivation and restriction, and metabolic blockers on food hoarding in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol. 1994;266:1111–7.
90. Boratyński JS, Jefimow M, Wojciechowski MS. Melatonin attenuates phenotypic flexibility of energy metabolism in a photoresponsive mammal, the Siberian hamster. J Exp Biol. 2017;220(Pt 17):3154–61.
91. Figala J, Hoffmann K, Goldau G. Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas. Oecologia. 1973;12:89–118.
92. Jefimow M, Przybylska-Piech AS, Wojciechowski MS. Predictive and reactive changes in antioxidant defence system in a heterothermic rodent. J Comp Physiol B. 2020;190(4):479–92.
93. Gutowski JP, Wojciechowski MS, Jefimow M. Diet affects resting, but not basal metabolic rate of normothermic Siberian hamsters acclimated to winter. Comp Biochem Physiol A Mol Integr Physiol. 2011;160(4):516–23.
94. Lighton JRB, Bartholomew GA, Feener DH. Energetics of locomotion and load carriage and a model of the energy-cost of foraging in the leaf-cutting ant Atta-Colombica Guer. Physiol Zool. 1987;60(5):524–37.
95. Koteja P. Measuring energy metabolism with open-flow Respirometric systems: which design to choose? Funct Ecol. 1996;10:675–7.
96. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67(1).
97. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2020. https://www.R-project.org/. Accessed Sept 2020.
98. Lenth R. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.6. ed: CRAN; 2020. p. https://CRAN.R-project.org/package=emmeans. Accessed September 2020.
99. Goodenough AE, Hart AG, Stafford R. Regression with empirical variable selection: description of a new method and application to ecological datasets. PLoS One. 2012;7(3):e34338.
100. Bartoń K. MuMIn: Multi-Model Inference R package version 1.43.17; 2020.

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5. Najważniejsze wyniki uzyskane w czasie badań

W czasie badań będących podstawą pracy doktorskiej uzyskałam wiele interesujących wyników. Najważniejsze z nich to:

- Chomiczniki wykazują powtarzalne różnice w zachowaniu oraz tempie metabolizmu, jednak zmiany w behawiorze zwierząt nie są związane ze zmianami ich podstawowego tempa metabolizmu (Artykuł I).
- Latem, przed wykształceniem zimowego fenotypu osobniki odpowiadające i nieodpowiadające na skracający się fotoperiod nie różnią się behawiorem oraz podstawowym tempem metabolizmu, ale osobniki odpowiadające charakteryzują się mniejszą elastycznością fenotypową sezonowych zmian zachowania i fizjologii (Artykuł I).
- Osobniki odpowiadające na skracający się fotoperiod przystępują do rozrodu później, a ich młode są mniejsze niż młode osobników nieodpowiadających (Artykuł II).
- Różnice w cechach historii życiowych między fenotypami nie wpływają na utrzymanie somatyczne zwierząt. Osobniki odpowiadające i nieodpowiadające na skracający się fotoperiod nie różnią się poziomem stresu oksydacyjnego i obrony antyoksydacyjnej oraz podstawowym tempem metabolizmu po zakończeniu reprodukcji (Artykuł II).
- Wydłużona aklimacja do krótkiego fotoperiodu zwiększa prawdopodobieństwo występowania osobników nie zmieniających koloru futra oraz osobników późno odpowiadających na krótki fotoperiod, ale nie wpływa na sezonowe zmiany masy ciała lub wykorzystanie dobowych odrętwień przez chomiczniki (Artykuł III).
- Polimorfizm zimowego fenotypu jest obserwowany nawet wewnątrz jednego miotu (Artykuł III).

6. Posumowanie i wnioski

Zjawisko polimorfizmu interesowało naukowców już w XIX wieku (Bateson, 1894; Doncaster, 1906). Wyróżniono wówczas dwa główne rodzaje polimorfizmu: przejściowy i zrównoważony (Ford, 1945). Pierwszy z nich obserwujemy jeśli nowy fenotyp pojawi się w populacji i dopiero się w niej rozprzestrzenia. Kiedy nowy, wyraźnie korzystniejszy i faworyzowany przez dobór naturalny fenotyp zastąpi fenotyp pierwotny, populacja znów będzie jednorodna. Z kolei występowanie w obrębie populacji kilku ustalonych, odmiennych fenotypów cechuje polimorfizm zrównoważony (Ford, 1945). Polimorfizm zimowego fenotypu jest przykładem polimorfizmu zrównoważonego (Heideman i Bronson, 1991; Moffatt et al., 1993; Nelson, 1987; Watson, 1962; Watson, 1973). Występowanie i utrzymywanie się w populacji chomiczników nieodpowiadających na krótki fotoperiod wskazuje, że wykształcenie takiego fenotypu może być korzystne. Nelson (1987) zaproponował, że dobór naturalny faworyzuje zmienność (różnorodność) cech, które są korzystne w zależności od warunków środowiska, przez co różne fenotypy mogą być utrzymywane w populacji. Z kolei w oparciu o badania Gormana i Zuckera (1997) postawiono hipotezę, że występowanie zimowego fenotypu jest zdeterminowane poligenowo, a polimorfizm jest skutkiem plejotropii antagonistycznej. Brak odpowiedzi na krótki fotoperiod z jednej strony zmniejsza prawdopodobieństwo przeżycia zimy, z drugiej jednak zwiększa sukces rozrodczy jeśli zwierzę zimę przeżyje (Prendergast et al., 2001). Inna hipoteza zakłada, że część osobników ma lepiej funkcjonujący układ immunologiczny, co pozwala im na przetrwanie zimy bez konieczności zmiany fenotypu na zimowy (Nelson et al., 1996). Jako podstawę polimorfizmu przedstawiono też hipotezę zakładającą niższe dostosowanie organizmu w warunkach typowych dla jego występowania i wyższe dostosowanie kiedy warunki są stresujące (Seger i Brockmann, 1987). Taka ryzykowna strategia może się opłacać w gwałtownie zmieniającym się środowisku. Wyniki uzyskane w moich pracach dostarczają nowych informacji i poszerzają istniejącą wiedzę na temat występowania oraz utrzymywania się polimorfizmu zimowego fenotypu wewnątrz populacji chomicznika dżungarskiego.

Chomicznik dżungarski jest gatunkiem modelowym zarówno w badaniach nad sezonowymi zmianami fenotypu zwierząt jak i nad polimorfizmem zimowego fenotypu

(Bao et al., 2019; Cubuk et al., 2016; Diedrich et al., 2020; Gorman i Zucker, 1995; Ruf i Heldmaier, 2000). Badania tego gatunku prowadzone są w warunkach laboratoryjnych, co umożliwiło dokładne poznanie odpowiedzi sezonowej chomiczników. Jednocześnie nie ma danych na temat polimorfizmu wśród chomiczników żyjących w warunkach naturalnych. Od lat pojawiają się pytania o to czy uzyskane w laboratoriach wyniki odpowiadają rzeczywistemu zjawisku obserwowanemu w przyrodzie. Sugeruje się, że fenotyp nieodpowiadający jest artefaktem wynikającym po pierwsze z ograniczonej puli genetycznej dostępnych zwierząt (Lynch et al., 1989; Nelson, 1985b), a po drugie ze stabilnych warunków występujących w laboratorium (Gorman et al., 1997; Prendergast et al., 2001). Choć nie ma wystarczających dowodów na występowanie w naturze chomiczników nieodpowiadających, to Kliman i Lynch (1992) podają, że już w trzecim pokoleniu chomiczników hodowanych w laboratorium obserwowano dużą proporcję osobników nieodpowiadających. Istnieje ponadto jedno doniesienie o chomicznikach dżungarskich rozmnażających się zimą w Kazachstanie (Kryltsov i Shubin, 1964), w którym autorzy wskazują, że w niektórych latach nawet 60% samic chomicznika rozmnaża się zimą. Sugeruje to że mogą stanowić one fenotyp nieodpowiadający i że międzyosobnicza zmienność cech zimowego fenotypu jest zjawiskiem występującym i utrzymywanym w naturze. W oparciu o uzyskane przeze mnie wyniki proponuję, że: 1) występowanie chomiczników nieodpowiadających nie jest artefaktem wynikającym z hodowli laboratoryjnej, a fenotyp nieodpowiadający może występować w naturze; 2) obecność tak wielu różnych zimowych fenotypów w populacji chomiczników jest przykładem zrównoważonego polimorfizmu; 3) gatunek ten jest doskonałym modelem do badań nad polimorfizmem zimowego fenotypu, zarówno w perspektywie badań laboratoryjnych, jak i tych przeprowadzanych w warunkach półnaturalnych czy naturalnych.

W swojej pracy stwierdziłam, że osobniki nieodpowiadające na krótki fotoperiod przystępują do rozrodu wcześniej i wydają na świat większe młode, jednocześnie nie wykazując wyższego poziomu stresu oksydacyjnego po zakończeniu rozmnażania niż osobniki o fenotypie odpowiadającym (Artykuł II). Chomiczniki nieodpowiadające mogą więc charakteryzować się większym sukcesem rozrodczym niż chomiczniki odpowiadające. Dodatkowo chomiczniki urodzone wcześnie w ciągu sezonu rozrodczego są dłużej eksponowane na działanie długiego fotoperiodu, co zmniejsza prawdopodobieństwo wykształcenia przez nie zimowego fenotypu (Artykuł III), a tym samym zwiększa możliwość utrzymania fenotypu nieodpowiadającego w populacji. Wcześniej urodzone młode będą też starsze kiedy nadejdzie zima, a wraz z wiekiem maleje produkcja melatoniny (Lerchl, 1994) oraz wrażliwość na nią (Horton i Yellon, 2001). Prowadzi to do utrzymywania aktywnych gonad nawet po aklimacji do krótkiego fotoperiodu, a w konsekwencji do wydłużenia okresu reprodukcyjnego i zwiększenia dostosowania osobników nieodpowiadających. Z kolei osobniki odpowiadające o zredukowanych wydatkach energetycznych (Artykuł III), ale też niższym sukcesie reprodukcyjnym (Artykuł II), występują w warunkach laboratoryjnych, mimo dostępu do pokarmu *ad libitum* oraz umiarkowanej temperatury otoczenia (~20°C). Wykształcenie zimowego fenotypu nie jest więc związane wyłącznie z surowymi warunkami, w których te zwierzęta żyją w naturze, a fenotyp nieodpowiadający nie jest artefaktem który pojawił się w odpowiedzi na warunki laboratoryjne. Pomimo wysokiej odziedziczalności zimowego fenotypu (Kliman i Lynch, 1992; Lynch et al., 1989), polimorfizm jest obserwowany nawet wewnątrz miotu (Artykuł III). Wyniki te są zgodne z hipotezą zrównoważonego polimorfizmu zakładającą, że to właśnie różnorodność fenotypów jest faworyzowana przez dobór naturalny (Ford, 1945; Prendergast et al., 2001). Występowanie polimorfizmu zimowego fenotypu w naturalnych populacjach wielu gatunków zwierząt (Mills et al., 2018; Nelson, 1987; Prendergast et al., 2001) jest wsparciem dla tej hipotezy.

Hipotezę zrównoważonego polimorfizmu umacnia również fakt, że nawet 10% chomiczników wykazuje późną odpowiedź na krótki fotoperiod (Artykuł III). Opóźnione wykształcenie cech zimowych wydaje się być niekorzystne dla zwierząt, jednak taki fenotyp może mieć przewagę selekcyjną kiedy weźmiemy pod uwagę obserwowane w ostatnich latach opóźnienie pojawienia się pokrywy śnieżnej jesienią (Callaghan et al., 2012) oraz zwiększenie prawdopodobieństwa mrozów i opadów śniegu późną wiosną (Zohner et al., 2020). Ocieplenie się klimatu i wynikające z niego zmiany w środowisku naturalnym stały się jednym z wiodących zagadnień rozważanych przez współczesnych ekologów. Ciągle przybywa danych o negatywnym wpływie zmian klimatycznych na populacje dzikich zwierząt (Almond et al., 2020; Mondor i Tremblay, 2010; Spooner et al., 2018). Procesy fizjologiczne zachodzące zgodnie z rytmem naturalnych zmian środowiska, wynikających ze zmian długości fotoperiodu są szczególnie wrażliwe na zmiany klimatu (Parmesan, 2007; Scranton i Amarasekare, 2017; Visser i Both, 2005). Do takich zjawisk, nazywanych zjawiskami fenologicznymi, należy zmiana fenotypu na zimowy. Kiedy warunki środowiska zmieniają się w sposób nieprzewidywalny, a zmiany fenologiczne nie są do nich dopasowane, dochodzi do niedopasowania fenologicznego (Miller-Rushing et al., 2010; Visser i Both, 2005; Zimova et al., 2016). Jest ono obserwowane od lat, a jednym z najlepszych jego przykładów jest nieodpasowanie koloru futra zwierząt do koloru tła (środowiska). Wykazano, że takie niedopasowanie może zwiększać śmiertelność, a co za tym idzie zmniejszać dostosowanie zwierząt (Atmeh et al., 2018; Durant et al., 2007; Zimova et al., 2016). Kiedy długość dnia staje się nieadekwatną miarą warunków środowiska, czynnikiem selekcyjnym staje się inny czynnik zewnętrzny, np. opady śniegu, niska temperatura otoczenia, czy dostępność pożywienia (Parmesan, 2007; Scranton i Amarasekare, 2017). Proponuję, że osobniki wykazujące opóźnioną zmianę fenotypu na zimowy zyskują przewagę w takiej sytuacji, dzięki czemu są utrzymywane w populacji.

Zarówno zachowanie chomiczników, jak i ich metabolizm były powtarzalne, jednak zmiany tych cech nie korelowały ze sobą (Artykuł I). Mnogość zmian zachodzących w fizjologii i behawiorze chomiczników w odpowiedzi na skracający się fotoperiod wskazuje na złożony mechanizm odpowiedzi sezonowej. Szlaki molekularne odpowiedzialne za różne cechy zimowego fenotypu działają niezależnie, ale są też ze sobą powiązane (Dardente et al., 2019; Dardente et al., 2010). Może to powodować występowanie w populacji całego spektrum osobników o częściowej odpowiedzi na krótki fotoperiod (Artykuły I i III). Odpowiedź sezonowa jest regulowana zmianą poziomu melatoniny wydzielanej przez szyszynkę. Jednocześnie melatonina jest sygnałem regulującym szlaki molekularne odpowiedzialne za zmiany poziomu hormonów wydzielanych przez przysadkę mózgową. Przykładowo, prolaktyna reguluje linienie, wzrost futra i zmianę jego koloru (Dardente, 2015; Duncan i Goldman, 1984a; Ebling, 2020). Podanie zwierzętom aklimowanym do krótkiego fotoperiodu egzogennej prolaktyny skutkuje zanikiem wielu cech zimowego fenotypu (Barrett et al., 2007; Duncan i Goldman, 1984b; Murphy et al., 2012; Ruby et al., 1993). Z kolei za gospodarkę energetyczną, metabolizm tłuszczów, aktywność brunatnej tkanki tłuszczowej, dobowe odrętwienia i reprodukcję odpowiadają m.in. hormony tarczycy. Podobnie jak w przypadku prolaktyny, egzogenne hormony tarczycy podane zwierzętom aklimowanym do krótkiego dnia wywołują zmianę fenotypu w kierunku letniego. Nie wiadomo, które z hormonów odpowiadają za sezonowe zmiany zachowania zwierząt czy tempa metabolizmu. Agresja oraz zachowania socjalne zwierząt zmieniają się głównie wraz ze zmianą stężenia hormonów steroidowych (Daan i Aschoff, 1975; Watts, 2020; Wilsterman et al., 2019). Z kolei zmiana tempa metabolizmu jest związana z gospodarką energetyczną, a więc z poziomem hormonów tarczycy (Dardente et al., 2019; Williams et al., 2017). Również prolaktyna może wpływać na tempo metabolizmu przez regulację pobierania pożywienia czy wytwarzania i magazynowania tkanki tłuszczowej (Ben-Jonathan et al., 2006; Yahata i Kuroshima, 1994). W oparciu o uzyskane dane sugeruję, że za zmiany w zachowaniu i metabolizmie zwierząt odpowiadają inne szlaki molekularne, co skutkuje niezależnymi zmianami behawioru i tempa metabolizmu oraz brakiem związku zachowania zwierząt z ich fenotypem zimowym.

Niemniej jednak, chomiczniki nieodpowiadające charakteryzują się większą sezonową elastycznością zachowania i podstawowego tempa metabolizmu (Artykuł I). Wcześniej stwierdzono, że wykazują one również większą elastyczność fenotypową podstawowego tempa metabolizmu w odpowiedzi na krótkie, nieprzewidywalne zmiany warunków środowiska (Boratyński et al., 2017a). Na podstawie uzyskanych wyników proponuję, że brak wykształcenia zimowego fenotypu u chomiczników nieodpowiadających może być kompensowany przez zwiększenie elastyczności innych cech, zarówno w kontekście zmian sezonowych, jak i nagłych, niecyklicznych zmian warunków środowiska. W efekcie zmian klimatycznych anomalie pogodowe są częstsze i bardziej gwałtowne w czasie zimy (Almond et al., 2020; Masson-Delmotte et al., 2018). W takich warunkach osobniki nieodpowiadające, o większej elastyczności fenotypowej będą uzyskiwały przewagę i dzięki temu będą utrzymywane w populacji. Z kolei osobniki odpowiadające charakteryzujące się odwracalnymi, sezonowymi zmianami fenotypu, mogą uzyskiwać przewagę selekcyjną w trakcie mroźnych i śnieżnych zim, w czasie których dostęp do pożywienia jest utrudniony.

Zmiany klimatyczne, w tym ocieplenie klimatu i łagodniejsze zimy mogą faworyzować osobniki o fenotypach nieodpowiadającym lub późno odpowiadającym, przyczyniając się do występowania i utrzymywania polimorfizmu zimowego fenotypu w populacji. Mills i współautorzy (2018) podają, że obecnie między 2 a 57% powierzchni zasięgu gatunków polimorficznych zajmują strefy, w których występują zarówno osobniki zmieniające, jak i nie zmieniające koloru futra na biały. Tak duży i stale powiększający się zasięg występowania gatunków polimorficznych potwierdza moje przewidywania, że różne fenotypy faworyzowane są przez dobór naturalny w innych warunkach i żaden nie uzyskuje ewolucyjnej przewagi nad pozostałymi. Uzyskane przeze mnie wyniki poszerzają wiedzę o samym zjawisku polimorfizmu zimowego fenotypu jak i poszczególnych fenotypach chomiczników dżungarskich. Porównując tempo metabolizmu, zachowanie i historie życiowe różnych fenotypów opisałam możliwe korzyści i koszty płynące z ich wykształcenia oraz zaproponowałam jakie warunki muszą być spełnione aby dany fenotyp był utrzymywany w populacji.

7. Bibliografia

Albon, S. D., Clutton-Brock, T. H. i Guinness, F. E. (1987). Early development and population density in red deer II Density-Independent Effects and Cohort Variation. *Journal of Animal Ecology* **56**, 69-81.

Almond, R. E. A., Grooten, M. i Petersen, T. (2020). Living Planet Report 2020 - Bending the curve of biodiversity loss. Gland, Switzerland: WWF.

Arnold, A. i Rubin, R. T. (2009). Mammalian Seasonal Rhythms: Behavior and Neuroendocrine Substrates. *Hormones, Brain and Behavior* **1**, 507-538.

Atmeh, K., Andruszkiewicz, A. i Zub, K. (2018). Climate change is affecting mortality of weasels due to camouflage mismatch. *Scientific Reports* **8**, 7648.

Bao, R., Onishi, K. G., Tolla, E., Ebling, F. J. P., Lewis, J. E., Anderson, R. L., Barrett, P., Prendergast, B. J. i Stevenson, T. J. (2019). Genome sequencing and transcriptome analyses of the Siberian hamster hypothalamus identify mechanisms for seasonal energy balance. *Proceedings of the National Academy of Sciences of the United States of America*.

Barrett, P., Ebling, F. J., Schuhler, S., Wilson, D., Ross, A. W., Warner, A., Jethwa, P., Boelen, A., Visser, T. J., Ozanne, D. M. et al. (2007). Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology* **148**, 3608-17.

Bartness, T. J. i Goldman, B. (1988). Peak duration of serum melatonin and short-day responses in adult Siberian hamsters. *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **255**, 812-822.

Bartness, T. J. i Goldman, B. D. (1989). Mammalian Pineal Melatonin - a Clock for All Seasons. *Experientia* **45**, 939-945.

Bateson, W. (1894). Materials for the study of variation treated with especial regard to discontinuity in the origin of species. London: Macmillan and co.

Beckerman, A. P., Benton, T. G., Ranta, E., Kaitala, V. i Lundberg, P. (2002). Population dynamic consequences of delayed life-history effects. *Trends in Ecology & Evolution* **17**, 263-269.

Ben-Jonathan, N., Hugo, E. R., Brandebourg, T. D. i LaPensee, C. R. (2006). Focus on prolactin as a metabolic hormone. *Trends in Endocrinology and Metabolism* **17**, 110-6.

Bernard, D. J., Losee-Olson, S. i Turek, F. W. (1997). Age-related changes in the photoperiodic response of Siberian hamsters. *Biology of Reproduction* **57**, 172-7.

Blank, J. L., Korytko, A. I. i Weber, S. (1992). Differential Response of Female Deer Mice to Short Photoperiod. *Ohio Journal of Science* **92**, 4-7.

Blank, J. L., Nelson, R. J., Vaughan, M. K. i Reiter, R. J. (1988). Pineal melatonin content in photoperiodically responsive and non-responsive phenotypes of deer mice. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **91**, 535-7.

Boratyński, J. S., Jefimow, M. i Wojciechowski, M. S. (2017a). Individual Differences in the Phenotypic Flexibility of Basal Metabolic Rate in Siberian Hamsters Are Consistent on Short- and Long-Term Timescales. *Physiological and Biochemical Zoology* **90**, 139-152.

Boratyński, J. S., Jefimow, M. i Wojciechowski, M. S. (2017b). Melatonin attenuates phenotypic flexibility of energy metabolism in a photoresponsive mammal, the Siberian hamster. *Journal of Experimental Biology* **220**, 3154-3161.

Bronson, F. H. i Perrigo, G. (1987). Seasonal Regulation of Reproduction in Muroid Rodents. *American Zoologist* **27**, 929-940.

Butler, M. P., Paul, M. J., Turner, K. W., Park, J. H., Driscoll, J. R., Kriegsfeld, L. J. i Zucker, I. (2008). Circadian rhythms of photorefractory siberian hamsters remain responsive to melatonin. *Journal of Biological Rhythms* **23**, 160-9.

Butler, M. P., Trumbull, J. J., Turner, K. W. i Zucker, I. (2007). Timing of puberty and synchronization of seasonal rhythms by simulated natural photoperiods in female Siberian hamsters. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **293**, R413-20.

Butler, M. P., Turner, K. W., Park, J. H., Schoomer, E. E., Zucker, I. i Gorman, M. R. (2010). Seasonal regulation of reproduction: altered role of melatonin under naturalistic conditions in hamsters. *Proceedings of the Royal Society B: Biological Sciences* **277**, 2867-74.

Careau, V., Montiglio, P.-O., Garant, D., Pelletier, F., Speakman, J. R., Humphries, M. M. i Réale, D. (2015). Energy expenditure and personality in wild chipmunks. *Behavioral Ecology and Sociobiology* **69**, 653-661.

Careau, V., Thomas, D., Humphries, M. M. i Reale, D. (2008). Energy metabolism and animal personality. *Oikos* **117**, 641-653.

Clutton-Brock, T. H., Major, M., Albon, S. D. i Guinness, F. E. (1987). Early Development and Population Dynamics in Red Deer. I. Density-Dependent Effects on Juvenile Survival. *Journal of Animal Ecology* **56**, 53-67.

Cubuk, C., Bank, J. H. i Herwig, A. (2016). The Chemistry of Cold: Mechanisms of Torpor Regulation in the Siberian Hamster. *Physiology (Bethesda)* **31**, 51-9.

Daan, S. i Aschoff, J. (1975). Circadian Rhythms of Locomotor Activity in Captive Birds and Mammals: Their Variations with Season and Latitude. *Oecologia* **18**, 269-316.

Dardente, H. (2015). Circannual Biology: The Double Life of the Seasonal Thyrotroph. *Current Biology* **25**, R988-91.

Dardente, H., Wood, S., Ebling, F. i Saenz de Miera, C. (2019). An integrative view of mammalian seasonal neuroendocrinology. *Journal of Neuroendocrinology* **31**, e12729.

Dardente, H., Wyse, C. A., Birnie, M. J., Dupre, S. M., Loudon, A. S., Lincoln, G. A. i Hazlerigg, D. G. (2010). A molecular switch for photoperiod responsiveness in mammals. *Current Biology* **20**, 2193-8.

Diedrich, V., Bank, J. H., Scherbarth, F. i Steinlechner, S. (2015). Torpor expression in juvenile and adult Djungarian hamsters (Phodopus sungorus) differs in frequency, duration and onset in response to a daily cycle in ambient temperature. *Journal of Thermal Biology* **53**, 23-32.

Diedrich, V., Haugg, E., Dreier, C. i Herwig, A. (2020). What can seasonal models teach us about energy balance? *Journal of Endocrinology* **244**, R17-R32.

Doncaster, L. (1906). On Breeding Experiments With Lepidoptera. *Proceedings of the Zoological Society of London* **76**, 125–133.

Duncan, M. J. i Goldman, B. (1984a). Hormonal Regulation of the Annual Pelage Color Cycle in the Djungarian Hamster , Phodopus sungorus. 1. Role of the Gonads and the Pituitary. *The Journal of Experimental Zoology* **230**, 89-95.

Duncan, M. J. i Goldman, B. D. (1984b). Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, Phodopus sungorus. II. Role of prolactin. *Journal of Experimental Zoology* **230**, 97-103.

Durant, J. M., Hjermann, D., Ottersen, G. i Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate Research* **33**, 271-283.

Ebling, F. (2020). Seasonal rhythms of energy metabolism. *Biological Clocks*.

Ergon, T., Lambin, X. i Stenseth, N. C. (2001). Life-history traits of voles in a fluctuating population repsond to immediate environment. *Nature* **411**, 1043-1045.

Figala, J., Hoffmann, K. i Goldau, G. (1973). Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas. *Oecologia* **12**, 89-118.

Ford, E. B. (1945). Polymorphism. *Biological Reviews of the Cambridge Philosophical Society* **20**, 73-88.

Freeman, D. A. i Goldman, B. D. (1997). Photoperiod nonresponsive Siberian hamsters: effect of age on the probability of nonresponsiveness. *Journal of Biological Rhythms* **12**, 110-21.

Goldman, S. L., Dhandapani, K. i Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (Phodopus sungorus). *Journal of Biological Rhythms* **15**, 417-28.

Goldman, S. L. i Goldman, B. D. (2003). Early photoperiod history and short-day responsiveness in Siberian hamsters. *Journal of Experimental Zoology Part A Comparative Experimental Biology* **296**, 38-45.

Gorman, M. R., Freeman, D. A. i Zucker, I. (1997). Photoperiodism in hamsters: abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. *J Biol Rhythms* **12**, 122-35.

Gorman, M. R. i Zucker, I. (1995). Seasonal adaptations of Siberian hamsters. II. Pattern of change in day length controls annual testicular and body weight rhythms. *Biology of Reproduction* **53**, 116-125.

Gorman, M. R. i Zucker, I. (1997). Environmental induction of photononresponsiveness in the Siberian hamster, Phodopus sungorus. *American Journal of Physiology* **272**, R887-95.

Heath, H. W. i Lynch, G. R. (1982). Intraspecific differences for melatonin-induced reproductive regression and the seasonal molt in Peromyscus leucopus. *General and Comparative Endocrinology* **48**, 289-95.

Heideman, P. D. i Bronson, F. H. (1991). Characteristics of a genetic polymorphism for reproductive photoresponsiveness in the white-footed mouse (Peromyscus leucopus). *Biology of Reproduction* **44**, 1189-96.

Heideman, P. D., Bruno, T. A., Singley, J. W. i Smedley, J. V. (1999). Genetic variation in photoperiodism in Peromyscus leucopus: Geographic variation in an alternative life-history strategy. *Journal of Mammalogy* **80**, 1232-1242.

Heldmaier, G. i Steinlechner, S. (1981). Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, Phodopus sungorus. *Oecologia* **48**, 265-270.

Helle, H., Koskela, E. i Mappes, T. (2012). Life in varying environments: experimental evidence for delayed effects of juvenile environment on adult life history. *Journal of Animal Ecology* **81**, 573-82.

Hoffmann, K. (1978). Effects of short photoperiods on puberty, growth and moult in the Djungarian hamster (Phodopus sungorus). *Journal of Reproduction and Fertility* **54**, 29-35.

Hoffmann, K. i Illnerova, H. (1986). Photoperiodic effects in the Djungarian hamster. Rate of testicular regression and extension of pineal melatonin pattern depend on the way of change from long to short photoperiods. *Neuroendocrinology* **43**, 317-21.

Horton, T. H. i Yellon, S. M. (2001). Aging, reproduction, and the melatonin rhythm in the Siberian hamster. *Journal of Biological Rhythms* **16**, 243-253.

Huck, U. W., Labov, j. B. i lisk, R. D. (1986). Food Restricting Young Hamsters (Mesocricetus auratus) Affects sex ration and growth of subsequent offspring. *Biology of Reproduction* **35**, 592-598.

Huxley, J. (1955). Morphism and Evolution. *Heredity* **9**, 1-52.

Jastroch, M., Giroud, S., Barrett, P., Geiser, F., Heldmaier, G. i Herwig, A. (2016). Seasonal Control of Mammalian Energy Balance: Recent Advances in the Understanding of Daily Torpor and Hibernation. *Journal of Neuroendocrinology* **28**.

Jefimow, M., Przybylska-Piech, A. S. i Wojciechowski, M. S. (2020). Predictive and reactive changes in antioxidant defence system in a heterothermic rodent. *Journal of Comparative Physiology B* **190**, 479-492.

Kliman, R. M. i Lynch, G. R. (1992). Evidence for genetic variation in the occurrence of the photoresponse of the Djungarian hamster, Phodopus sungorus. *Journal of Biological Rhythms* **7**, 161-73.

Kryltsov, A. I. i Shubin, I. G. (1964). On The Ecology of Cricetulus Eversmanni Br. and Phodopus Sungorus Pall. *ZOOLOGICHESKIY ZHURNAL* **43**, 1062-1069.

Leimar, O. (2005). The evolution of phenotypic polymorphism: randomized strategies versus evolutionary branching. *American Naturalist* **165**, 669-81.

Lerchl, A. (1994). Increased oxidation of pineal serotonin as a possible explanation for reduced melatonin synthesis in the aging Djungarian hamster (Phodopus sungorus). *Neuroscience Letters* **176**, 25-28.

Levesque, D. L. i Tattersall, G. J. (2010). Seasonal torpor and normothermic energy metabolism in the Eastern chipmunk (Tamias striatus). *Journal of Comparative Physiology B* **180**, 279-92.

Li, X. S. i Wang, D. H. (2005). Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils (Meriones unguiculatus): the roles of short photoperiod and cold. *Journal of Comparative Physiology B* **175**, 593-600.

Lynch, G. R. (1973). Effect of simultaneous exposure to differences in photoperiod and temperature on the seasonal molt and reproductive system of the white-footed mouse, Peromyscus leucopus *Comparative Biochemistry and Physiology* **44**, 1373-1376.

Lynch, G. R., Lynch, C. B. i Kliman, R. M. (1989). Genetic analyses of photoresponsiveness in the Djungarian hamster, Phodopus sungorus. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **164**, 475-81.

Łomnicki, A. (2009). Dobor naturalny. Dostosowanie i współczynnik doboru oraz inne miary dostosowania. *Kosmos* **58**, 323-328.

Majoy, S. B. i Heideman, P. D. (2000). Tau Differences between Short-Day Responsive and Short-Day Nonresponsive White-Footed Mice (Peromyscus leucopus) Do Not Affect Reproductive Photoresponsiveness. *Journal of Biological Rhythms* **15**, 500-512.

Margraf, R. R., Zlomanczuk, P., Liskin, L. A. i Lynch, G. R. (1991). Circadian differences in neuronal activity of the suprachiasmatic nucleus in brain slices prepared from photo-responsive and

photo-non-responsive Djungarian hamsters. *Brain Research* **544**, 42-48.

Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., Shukla, P. R., Pirani, A., Moufouma-Okia, W., Péan, C., Pidcock, R. et al. (2018). Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty: IPCC.

Miller-Rushing, A. J., Hoye, T. T., Inouye, D. W. i Post, E. (2010). The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 3177-86.

Mills, L. S., Bragina, E. V., Kumar, A. V., Zimova, M., Lafferty, D. J. R., Feltner, J., Davis, B. M., Hacklander, K., Alves, P. C., Good, J. M. et al. (2018). Winter color polymorphisms identify global hot spots for evolutionary rescue from climate change. *Science* **359**, 1033-1036.

Moffatt, C. A., Devries, A. C. i Nelson, R. J. (1993). Winter Adaptations of Male Deer Mice (Peromyscus-Maniculatus) and Prairie Voles (Microtus-Ochrogaster) That Vary in Reproductive Responsiveness to Photoperiod. *Journal of Biological Rhythms* **8**, 221-232.

Mondor, E. i Tremblay, M. (2010). Global Atmospheric Change and Animal Populations. *Nature Education Knowledge* **3**, 23.

Murphy, M., Jethwa, P. H., Warner, A., Barrett, P., Nilaweera, K. N., Brameld, J. M. i Ebling, F. J. (2012). Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinology* **153**, 101-12.

Nelson, R. J. (1985a). Photoperiod influences reproduction in the prairie vole (Microtus ochrogaster). *Biology of Reproduction* **33**, 596-602.

Nelson, R. J. (1985b). Photoperiodic regulation of Reproductive Development in Male Praire Voles: Influence of Laboratory Breeding. *Biology of Reproduction* **33**, 418-422.

Nelson, R. J. (1987). Photoperiod-Nonresponsive Morphs: A Possible Variable in Microtine Population-Density Fluctuations. *The American Naturalist* **130**, 350-369.

Nelson, R. J., Fine, J. B., Demas, G. E. i Moffatt, C. A. (1996). Photoperiod and population density interact to affect reproductive and immune function in male prarie voles. *American journal of Physiology* **270**, 571-577.

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology* **13**, 1860-1872.

Petri, I., Diedrich, V., Wilson, D., Fernandez-Calleja, J., Herwig, A., Steinlechner, S. i Barrett, P. (2016). Orchestration of gene expression across the seasons: Hypothalamic gene expression in natural photoperiod throughout the year in the Siberian hamster. *Scientific Reports* **6**, 29689.

Piersma, T. i Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology & Evolution* **18**, 228-233.

Piersma, T. i Van Gils, J. A. (2011). The flexible phenotype. Oxford: Oxford.

Place, N. J. i Cruickshank, J. (2009). Graded response to short photoperiod during development and early adulthood in Siberian hamsters and the effects on reproduction as females age. *Hormones and Behavior* **55**, 390-7.

Place, N. J., Tuthill, C. R., Schoomer, E. E., Tramontin, A. D. i Zucker, I. (2004). Short day lengths delay reproductive aging. *Biology of Reproduction* **71**, 987-92.

Prendergast, B. J., Kriegsfeld, L. J. i Nelson, R. J. (2001). Photoperiodic polyphenisms in rodents: neuroendocrine mechanisms, costs, and functions. *The Quarterly Review of Biology* **76**, 293-325.

Prevot-Julliard, A. C., Henttonens, H., Yoccoz, N. G. i Stenseth, N. C. (1999). Delayed maturation in female bank voles: optimal decision or social constraint? *Journal of Animal Ecology* **68**, 684-697.

Przybylska-Piech, A. S., Wojciechowski, M. S. i Jefimow, M. (2021). Consecutive litters do not differ in photoresponsiveness but prolonged acclimation to long photoperiod inhibits winter molt. *Frontiers in Zoology*.

Przybylska, A. S., Wojciechowski, M. S. i Jefimow, M. (2019a). Photoresponsiveness affects life history traits but not oxidative status in a seasonal rodent. *Frontiers in Zoology* **16**, 11.

Przybylska, A. S., Wojciechowski, M. S. i Jefimow, M. (2019b). Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behaviour. *Animal Behaviour* **147**, 105-114.

Puchalski, W. i Lynch, G. R. (1986). Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. *Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology* **159**, 7-11.

Puchalski, W. i Lynch, G. R. (1988). Characterization of circadian function in Djungarian hamsters insensitive to short day photoperiod. *Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology* **162**, 309-16.

Puchalski, W. i Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreatment and artificial selection. *American Journal of Physiology* **261**, 670-676.

Reale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. i Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 4051-63.

Rogovin, K. A., Bushuev, A. V., Khruscheva, A. M. i Vasilieva, N. Y. (2014). Resting metabolic rate, stress, testosterone, and induced immune response in spring- and fall-born males of Campbell's dwarf hamsters: Maintenance in long-day conditions. *Biology Bulletin Reviews* **4**, 181-191.

Ruby, N. F., Nelson, R. J., Licht, P. i Zucker, I. (1993). Prolactin and testosterone inhibit torpor in Siberian hamsters. *American Journal of Physiology* **264**, R123-8.

Ruf, T., Bieber, C., Arnold, W. i Millesi, E. (2012a). Living In A Seasonal World. Thermoregulatory and Metabolic Adaptations. Berlin Heidelberg: Springer.

Ruf, T., Bieber, C. i Turbill, C. (2012b). Survival, Aging, and Life-History Tactics in Mammalian Hibernators. In *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*, eds. T. Ruf C. Bieber W. Arnold and E. Millesi), pp. 123-132. Berlin, Heidelberg: Springer Berlin Heidelberg.

Ruf, T. i Heldmaier, G. (2000). Djungarian Hamsters - Small Graminivores with Daily Torpor. In *Activity Patterns in Small Mammals: An Ecological Approach*, eds. S. Halle and N. C. Stenseth). Berlin: Springer.

Ruf, T., Stieglitz, A., Steinlechner, S., Blank, J. L. i Heldmaier, G. (1993). Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (Phodopus sungorus). *Journal of Experimental Zoology* **267**, 104-12.

Schmidt-Nielsen, K. (2008). Fizjologia zwierząt. Adaptacja do Środowiska. Warszawa: Wydawnictwo naukow PWN.

Scranton, K. i Amarasekare, P. (2017). Predicting phenological shifts in a changing climate. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 13212-13217.

Searcy, C. A., Gray, L. N., Trenham, P. C. i Shaffer, H. B. (2014). Delayed life history effects, multilevel selection, and evolutionary trade-offs in the California tiger salamander. *Ecology* **95**, 68-77.

Seger, J. i Brockmann, H. J. (1987). What is Bet-Hedging. In *Oxford Surveys in Evolutionary Biology*, vol. 4 eds. P. H. Harvey and L. Partridge): Oxford University Press.

Severaid, J. H. (1945). Pelage Changes in the Snowshoe Hare (Lepus-Americanus-Struthopus Bangs). *Journal of Mammalogy* **26**, 41-63.

Spooner, F. E. B., Pearson, R. G. i Freeman, R. (2018). Rapid warming is associated with population decline among terrestrial birds and mammals globally. *Global Change Biology* **24**, 4521-4531.

Steinlechner, S. i Heldmaier, G. (1982). Role of photoperiod and melatonin in seasonal acclimatization of the Djungarian hamster, Phodopus sungorus. *International Journal of Biometeorology* **26**, 329-37.

Steinlechner, S., Heldmaier, G. i Becker, H. (1983). The seasonal cycle of body weight in the Djungarian hamster: photoperiodic control and the influence of starvation and melatonin. *Oecologia* **60**, 401-405.

Turbill, C., Smith, S., Deimel, C. i Ruf, T. (2012). Daily torpor is associated with telomere length change over winter in Djungarian hamsters. *Biology Letters* **8**, 304-7.

Visser, M. E. i Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences* **272**, 2561-9.

Watson, A. (1962). The effect of climate on the colour changes of mountian hares in Scotland. *Proceedings of the Zoological Society of London.* **141**, 823–835.

Watson, A. (1973). Molts of Wild Scottish Ptarmigan, Lagopus-Mutus, in Relation to Sex, Climate and Status. *Journal of Zoology* **171**, 207-223.

Watts, H. E. (2020). Seasonal regulation of behaviour: what role do hormone receptors play? *Proceedings of the Royal Society B: Biological Sciences* **287**, 20200722.

Williams, C. T., Klaassen, M., Barnes, B. M., Buck, C. L., Arnold, W., Giroud, S., Vetter, S. G. i Ruf, T. (2017). Seasonal reproductive tactics: annual timing and the capital-to-income breeder continuum. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**.

Wilsterman, K., McGuire, N. L., Calisi, R. M. i Bentley, G. E. (2019). Seasonality: Hormones and Behavior. In *Encyclopedia of Animal Behavior*, pp. 599-612.

Wood, S. i Loudon, A. (2014). Clocks for all seasons: unwinding the roles and mechanisms of circadian and interval timers in the hypothalamus and pituitary. *Journal of Endocrinology* **222**, R39-59.

Yahata, T. i Kuroshima, A. (1994). Role of Prolactin in Brown Adipose Tissue Thermogenic Activity. In *Temperature Regulation Recent Pgysiological and Pharmacological Advances*, (ed. A. S. Milton), pp. 327-331: Springer Basel AG.

Zimova, M., Hacklander, K., Good, J. M., Melo-Ferreira, J., Alves, P. C. i Mills, L. S. (2018). Function and underlying mechanisms of seasonal colour moulting in mammals and birds: what keeps them changing in a warming world? *Biological Reviews of the Cambridge Philosophical Society*.

Zimova, M., Mills, L. S., Lukacs, P. M. i Mitchell, M. S. (2014). Snowshoe hares display limited phenotypic plasticity to mismatch in seasonal camouflage. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20140029.

Zimova, M., Mills, L. S. i Nowak, J. J. (2016). High fitness costs of climate change-induced camouflage mismatch. *Ecology Letters* **19**, 299-307.

8. Oświadczenia współautorów

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Oświadczam, że mój udział w realizacji pracy:

- **Przybylska A. S**., Wojciechowski M. S. , Jefimow M. (2019) Physiological differences between winter phenotypes of Siberian hamsters do not correlate with their behavior, Animal Behaviour 147:105-114; doi:10.1016/j.anbehav.2018.11.007 (Artykuł I) polegał na udziale w planowaniu badań, współprowadzeniu doświadczeń, analizie, interpretacji i opisie uzyskanych wyników oraz udziale w pisaniu manuskryptu.

- **Przybylska A. S.** , Wojciechowski M. S. , Jefimow M. (2019) Photoresponsiveness affects life history traits but not oxidative status in a seasonal rodent, Frontiers in Zoology 16:11; doi:10.1186/s12983-019-0311-3 (Artykuł II) polegał na udziale w planowaniu badań, współprowadzeniu doświadczeń, analizie i interpretacji uzyskanych wyników oraz pisaniu manuskryptu.

- **Przybylska-Piech A. S**. , Wojciechowski M. S. , Jefimow M. (2021) Polymorphism of winter phenotype in Siberian hamster: Consecutive litters do not differ in photoresponsiveness but prolonged acclimation to long photoperiod inhibits winter molt, Frontiers in Zoology; 10.1186/s12983-021-00391-3 (Artykuł III) polegał na stworzeniu koncepcji badań, pozyskaniu finansowania badań, kierowaniu projektem badawczym, planowaniu i prowadzeniu doświadczeń, analizie i interpretacji uzyskanych wyników oraz pisaniu manuskryptu.

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Jednocześnie wyrażam zgodę na przedłożenie powyższych publikacji jako rozprawę doktorską mgr Anny Przybylskiej-Piech.

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