Dynamic spleen mass in wild and domestic American mink

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The immune system is considered to be an energetically expensive component of an individual's life history. Investment in the immune system can depend on the environment that an individual finds itself in. The American mink, *Neovison vison*, exists in the natural environment and on fur farms. The natural environment and mink farm differ in many ways, such as wild mink being exposed to many parasites that are less prevalent and less abundant in the domestic environment because of veterinary care. We collected free-ranging mink from commercial trappers and domestic mink from fur farmers in Ontario and Nova Scotia, Canada, and examined relative spleen mass. Wild male mink had larger spleens than domestic mink in Nova Scotia, with a similar trend in Ontario. Female mink that escaped from farms (feral) in Nova Scotia had significantly larger spleens than their domestic counterparts on the farms. Both of these results are consistent with the prediction that the natural environment contains parasites and pathogens that require enhanced investment in the immune system. In Nova Scotia, females had larger spleens than males, whether considering wild or domestic populations. Finally, wild mink showed greater condition dependence of spleen mass than domestic populations. Further investigations should include experimental approaches such as providing veterinary care to wild populations to assess the effects of parasites and pathogens on the immune system. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 624–631.

ADDITIONAL KEYWORDS: condition dependence – disease – feral mink – fur farm – immune system – parasite.

INTRODUCTION

Resources are finite, and life-history theory argues that organisms must allocate energy to competing demands, including maintenance, growth, and reproduction (Roff, 1992, but see van Noordwijk & de Jong, 1986). The immune system and defence against pathogens are recognized as important and energetically demanding components of an organism's life history (Sheldon & Verhulst, 1996; Martin, Scheurlein & Wikelski, 2003; Martin *et al.*, 2006). The use of the size of immune system organs as an index of investment in the immune system is a common approach, and the spleen is of particular interest. The spleen is a relatively small but critical organ that is involved in the production of lymphocytes that are used to fight infection (John, 1994).

The mammalian spleen plays a role both in terms of the immune system and as a reservoir for red blood cells. Nonetheless, Corbin *et al.* (2008) concluded that spleen mass in red deer (*Cervus elaphus*) was an accurate measure of immune activity. The use of the size of the spleen as a proxy measure of immunological activity has been widespread, particularly in birds (Møller 1997; Møller *et al.*, 1998a; Shutler, Alisauskas & McLaughlin, 1999) and mammals (Cowan *et al.*, 2009; Schulte-Hostedde & Elsasser, 2011), under the assumption that a larger spleen produces and stores

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more lymphocytes than a smaller spleen (Nunn, 2002). Variation in the size of the spleen may be the result of variation in energy reserves (i.e. the spleen may be condition dependent) (Møller *et al*., 1998a; Vicente, Perez-Rodriguez & Gortazar, 2007; Luttermann & Bennett, 2008). Alternatively, spleens may be enlarged in parasitized individuals, and thus the effect of parasites may be greater than the effect of individual energetics. Spleen size has been positively associated with parasite load, whether measured as parasite richness (Morand & Poulin, 2000; Schulte-Hostedde & Elsasser, 2011), parasite intensity (Lefebvre *et al*., 2004) or observed in a parasite removal experiment (Brown & Brown, 2002).

Underlying the evolutionary ecology of the immune system is the observation that males are generally more heavily infected with parasites, especially in mammals (Schalk & Forbes, 1997, Moore & Wilson, 2002). This bias in parasitism may be the result of sexual selection in which males that compete intensely for mates may be at a higher risk of infection (Moore & Wilson, 2002; Owens, 2002; Klein, 2004), perhaps due to the immunosuppressive effects of testosterone (Folstad & Karter, 1992; Klein, 2004). These sex differences in parasitism are predicted to affect the size of the spleen (Cowan *et al*., 2009, Schulte-Hostedde & Elsasser, 2011) – if individuals with more intense parasitism rates have larger spleens, then males should have relatively larger spleens than females.

The domestic environment is typically quite distinct from a natural context. Predators are reduced or absent, food and other resources are abundant, and domestic animals are often vaccinated or medicated against parasites and pathogens. By definition, domestic populations are also under artificial selection because the breeder selects which individuals will breed to achieve specific phenotypes for human use (Price, 1984; Snyder *et al*., 1996; Belliveau *et al*., 1999). Here we test several hypotheses related to variation in spleen mass (and by extension immune system investment) in wild, domestic, and feral American mink. The American mink (*Neovison vison*) is a semi-aquatic member of the Mustelidae that is endemic to North America (Larivière, 1999). The species has been trapped for centuries for fur, and has been successfully domesticated since the late 1800s (Joergensen, 1985). Feral mink populations have been established throughout Europe and North America following the accidental and deliberate release of domestic mink from farms (Joergensen, 1985; Lode′, Cormier & Le Jaques, 2001; Kruska & Sidorovich, 2003; Reynolds, Short & Leigh, 2004; Bowman *et al*., 2007; McDonald, O'Hara & Morrish, 2007; Kidd *et al*., 2009). Although the ecological and genetic consequences of such releases have received much recent attention (e.g. Bonesi & Palazon, 2007; Bowman *et al*., 2007; Bowman & Schulte-Hostedde, 2009; Kidd *et al*., 2009; Nituch *et al*., 2011), there has been little understanding of the consequences of domestication and escape on the evolutionary ecology of the immune system. American mink can be infected by a variety of parasites, including a sinus worm (*Skrjabingylus nasicola*) and a Guinea worm (*Dracunculus insignis*; Linscombe, Kinler & Auerlich, 1982). We hypothesized that processes that occur in the environment will be different from those that occur in the domestic context, and specifically that patterns of variation in spleen mass would be distinct between wild and domestic mink. First, we predicted that domestic mink would have relatively smaller spleens than wild mink because domestic mink are raised in the farm environment where they are medicated and vaccinated against parasites and pathogens (Joergensen, 1985; Agriculture Canada 1988) and thus would not experience the same degree of infection as wild mink. Second, we predicted that wild male mink would have larger spleens than wild female mink, but that this pattern would not exist in domestic mink because of the relative lack of parasites and pathogens on the farms. Third, we predicted that condition dependence of spleen size would be absent in domestic mink because the energetic demands of maintenance, growth, and reproduction are mitigated by abundant food resources and thermally favourable housing on mink farms. Finally, we predicted that feral mink (escaped domestic mink) would have larger spleens than domestic mink on the farm because of the increased diversity and abundance of parasites they encounter in the natural environment (Morand & Poulin, 2000; Schulte-Hostedde & Elsasser, 2011).

METHODS

Free-ranging mink were obtained from fur harvesters across the provinces of Ontario and Nova Scotia, Canada. Captive domestic mink were obtained from fur farmers in Ontario and Nova Scotia. Mink carcasses were skinned by the harvesters or farmers, and subsequently weighed, necropsied, and spleens were excised and weighed (Acculab Vicon 303 pan scale). Measurements taken included carcass mass and snout–vent length (SVL).

To identify if free-ranging mink were escaped domestic mink (feral) or wild mink, we genotyped and assigned each individual to population clusters (domestic, hybrid, and wild) according to methods described by Kidd *et al*. (2009). Individuals were assigned to populations with a minimum membership probability of $q \geq 0.80$ (Kidd *et al*., 2009; Nituch *et al*., 2011). We excluded hybrid mink and juveniles from our analyses.

Table 1. Mean relative spleen mass, standard error and sample size for domestic, wild and feral mink from Ontario (ON) and Nova Scotia (NS)

Genotype	Province	Sex	N	Mean relative spleen mass	SE
Domestic	ON	F	27	0.911	0.070
		M	40	0.593	0.027
	NS	F	100	0.582	0.014
		M	100	0.381	0.013
Wild	ON	F	7	0.787	0.036
		M	26	0.664	0.035
	NS	F	1	0.846	–
		M	16	0.727	0.035
Feral	ON	F	1	0.625	–
		M	1	0.364	–
	NS	F	36	0.916	0.044
		M	2	0.715	0.013

STATISTICAL ANALYSES

Relative spleen mass was calculated as the proportion of skinned body mass composed of wet spleen mass. Thus, wet spleen mass was subtracted from skinned body mass, and relative spleen mass was calculated as spleen mass divided by spleen-less skinned body mass multiplied by 100.

We tested our hypotheses where sample sizes were appropriate. As noted in Table 1, sample sizes were very low generally for wild females, and feral males and females in Ontario. Thus, not all hypotheses could be tested using data from both provinces or with both sexes.

PREDICTION 1 – WILD MINK HAVE LARGER SPLEENS THAN DOMESTIC MINK

To test this prediction, we were limited to using male mink because of low sample sizes of females in one of these two categories for each of Ontario and Nova Scotia. We used a factorial ANOVA, using wild/domestic and province as independent factors, and relative spleen mass as the dependent variable.

PREDICTION 2 – FERAL DOMESTIC MINK HAVE LARGER SPLEENS THAN DOMESTIC MINK ON FARMS

We were restricted to using female mink in Nova Scotia for testing this prediction. We used a one-way ANOVA to compare relative spleen mass between feral and domestic mink.

PREDICTION 3 – MALE WILD MINK HAVE LARGER SPLEENS THAN FEMALE WILD MINK, BUT THERE IS NO DIFFERENCE BETWEEN MALE AND FEMALE DOMESTIC MINK

For each group of mink (wild and domestic), we used a factorial ANOVA using province and sex as independent factors, and relative spleen mass as the dependent variable. We were restricted to using wild mink from Ontario to test for differences between the sexes in wild mink because of a low sample size of wild female mink in Nova Scotia ($N = 1$).

PREDICTION 4 – CONDITION OF DEPENDENCE OF SPLEEN SIZE IN WILD MINK, BUT NOT DOMESTIC MINK

To assess condition dependence of spleen mass across multiple groups, ANCOVA is a typical approach, but one that assumes homogeneous slopes (i.e. that the slope from the regression between body mass and SVL is equal among all groups). To test this assumption, we conducted an ANCOVA among groups with an appropriate sample size ($N > 10$) using body mass as an independent variable, SVL as a continuous predictor, and group identity as a categorical factor.

We tested whether spleen mass was associated with body condition by using a multiple regression with both SVL and body mass as independent factors and spleen mass as the dependent variable (Schulte-Hostedde & Elsasser, 2011). We interpreted the partial correlation coefficient of body mass as the independent effect of body mass on spleen mass, after correcting for SVL. By definition, body condition is body mass corrected for body size (Schulte-Hostedde *et al.*, 2005). We compared the partial *r*-values between wild and domestic mink using pairwise comparisons (Zar, 1999).

RESULTS

We collected data on spleen mass and body size and mass from 357 mink carcasses (102 from Ontario, 255 from Nova Scotia).
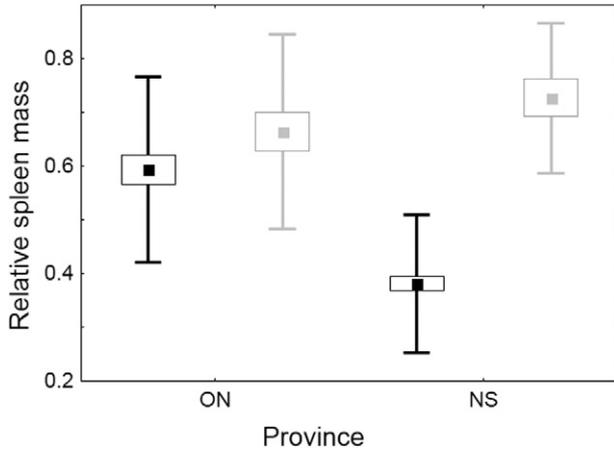
Figure 1. Relative spleen mass of male domestic (black) and wild (grey) mink from Ontario (ON) and Nova Scotia (NS). Wild mink have significantly larger spleens than domestic mink in Nova Scotia ($P < 0.001$). In Ontario, there is a similar trend ($P = 0.12$). Means, standard errors (box), and standard deviations (whisker) are presented.
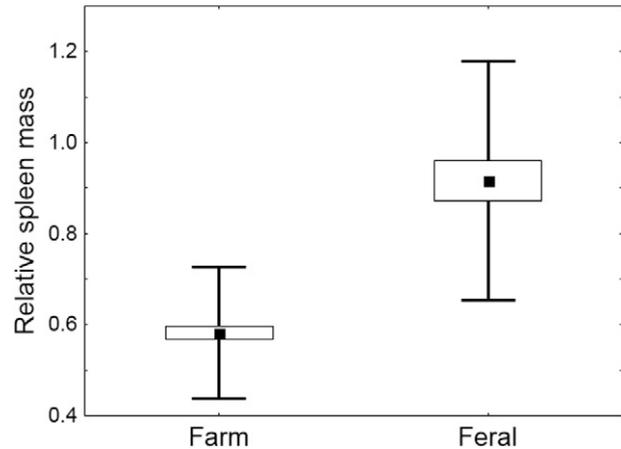

Figure 2. Spleen mass of female domestic mink on farms and feral domestic mink from Nova Scotia. Feral mink have larger spleens than domestic mink ($P < 0.001$), indicating an increase in spleen size when domestic mink escape into the natural environment. Means, standard errors (box), and standard deviations (whisker) are presented.

PREDICTION 1 – WILD MINK HAVE LARGER SPLEENS THAN DOMESTIC MINK

Among male mink, we found a significant interaction between province and genotype ($F_{1,178} = 25.40$, $P < 0.001$; Fig. 1), and we therefore analysed each province separately. In Nova Scotia, wild male mink had larger spleens than domestic males ($F_{1,114} = 97.31$, $P < 0.001$) whereas in Ontario, there was no significant difference in relative spleen mass between domestic and wild male mink ($F_{1,64} = 2.50$, $P = 0.12$) although the pattern was in the same direction as in Nova Scotia.

PREDICTION 2 – FERAL DOMESTIC MINK HAVE LARGER SPLEENS THAN DOMESTIC MINK ON FARMS

In Nova Scotia, feral female mink had significantly larger spleens than domestic females on the farm ($F_{1,134} = 88.96$, $P < 0.001$) (Fig. 2).

PREDICTION 3 – MALE WILD MINK HAVE LARGER SPLEENS THAN FEMALE WILD MINK, BUT THERE IS NO DIFFERENCE BETWEEN MALE AND FEMALE DOMESTIC MINK

In Ontario, wild female mink had larger spleens, although the difference was not significant ($F_{1,31} = 2.98$, $P = 0.094$). In domestic mink, there was a significant sex × province interaction ($F_{1,263} = 5.34$, $P = 0.02$) (Fig. 3), and thus we examined each province independently. In Ontario, female domestic mink had larger spleens than males ($F_{1,65} = 22.85$, $P < 0.001$), and

in Nova Scotia, females also had larger spleens than males ($F_{1,198} = 107.7$, $P < 0.001$).

PREDICTION 4 – CONDITION OF DEPENDENCE OF SPLEEN SIZE IN WILD MINK, BUT NOT DOMESTIC MINK

We could not include all male domestic mink from Ontario in our analysis of condition dependence because a subset ($N = 8$) did not have a measure of body length. Thus the sample size for this group is reduced in the analysis relative to the total number of males from Ontario farms for which we had data on spleen mass.

The assumption of identical slopes between body mass and body size among the groups of mink was not upheld (overall model $F_{13,324} = 204.4$, $P < 0.001$, Group × SVL $F_{6,324} = 8.06$, $P < 0.001$) and thus we assessed condition dependence of spleen mass for each of the groups (wild/domestic, male/female).

All groups of mink showed evidence of condition-dependence of relative spleen mass (Table 2). No pairwise comparisons of partial-correlation coefficients between wild and domestic mink were significantly different ($P > 0.1$ for all comparisons). Nonetheless, using a t-test to compare the mean values of the partial correlation coefficients between wild and domestic male mink [using Ontario and Nova Scotia values ($n = 2$) for each group], wild mink showed greater condition dependence than domestic males ($t = 7.47$, d.f. = 2, $P = 0.017$).

DISCUSSION

Relative spleen mass is expected to respond to both individual energetics, and the presence of parasites
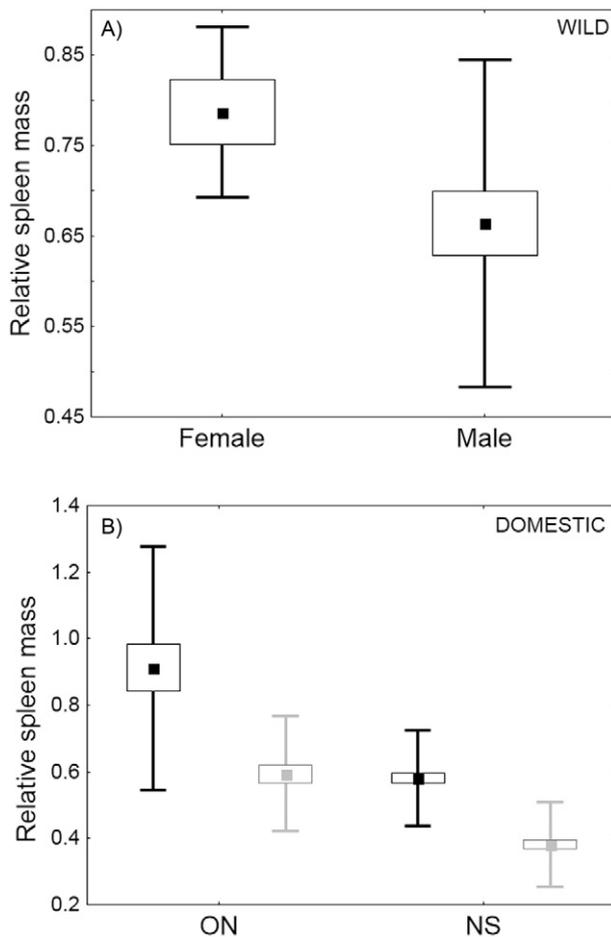

Figure 3. Sex differences in relative spleen mass in wild and domestic mink. In wild mink in Ontario (A), female mink tend to have larger spleens than males ($P = 0.094$). In domestic mink, females have larger spleens than males in both Ontario (ON, black) and Nova Scotia (NS, grey) ($P < 0.001$ for both provinces). Means, standard errors (box), and standard deviations (whisker) are presented.

(Møller *et al.*, 1998a; Morand & Poulin, 2000; Cowan *et al.*, 2009; Schulte-Hostedde & Elsasser, 2011). American mink exist in both the wild and in a domestic context, providing a unique opportunity to examine how spleen mass is affected by both the natural and the farm environment. Our results indicated significant differences in relative spleen mass between wild and domestic mink, with wild mink having larger spleens than domestic mink. This apparent effect of the natural environment on the immune system is supported by the observation that when domestic mink escape and become feral, their spleens enlarge. These results are interesting because they are consistent with the hypothesis that the relatively parasite-free environment imposed on domestic mink leads to differences in immune system investment relative to wild populations. One perspective is that the evident differences in spleen mass between wild and domestic mink may be the result of genetic differences between two populations. Domestic mink have been subject to intense artificial selection and this may have had consequences for immune system investment, leading to differences in spleen mass [assuming spleen mass is heritable (Stevens, 1991)]. Nonetheless, data from feral and domestic female mink indicate that spleen mass is dynamic and may respond to differences in the environment. One difference between the farm and natural environment may be exposure to pathogens and parasites. Domestic mink on mink farms often receive some form of veterinary care and enhanced biosecurity, including monitoring of health, vaccination, treatment (for parasites, disease, and injury), and quarantine where appropriate. The presence of parasites [particularly the diversity of endoparasites in mink (Schulte-Hostedde & Elsasser, 2011)] is positively associated with spleen size, and so the dynamic enlargement of spleen size found in feral mink may be the result of the novel exposure to parasites in the natural environment. Mink are subject to a variety of parasites in the natural environment (Linscombe *et al.*, 1982) that are presumably absent from the domestic context.

Table 2. Partial correlation coefficients of body mass corrected for body length (body condition) on relative spleen mass

	Partial r – body mass	P	Overall model r^2	Overall model P
NS domestic female ($N = 100$)	0.453	< 0.001	0.368	< 0.001
ON domestic female ($N = 27$)	0.538	0.005	0.291	0.016
NS domestic male ($N = 100$)	0.377	< 0.001	0.150	< 0.001
ON domestic male ($N = 32$)	0.356	0.050	0.333	0.003
NS wild male ($N = 16$)	0.583	0.022	0.397	0.037
ON wild male ($N = 26$)	0.646	< 0.001	0.522	< 0.001

All groups of mink show evidence of positive condition-dependence of spleen size. NS, Nova Scotia; ON, Ontario.

Exposure to these parasites may lead to enhanced immunological activity and thus an enlarged spleen. One complicating factor is the presence of Aleutian Disease Virus (ADV), a lethal parvovirus that afflicts domestic mink on mink farms (Nituch *et al.*, 2011). Nonetheless, there is no evidence that ADV infection is associated with enlarged spleens in wild mink (our unpublished data).

A general pattern among mammals is that males tend to have more parasites and pathogens than females (Moore & Wilson, 2002). Males are thought to have less effective immune systems, in part because of the immunosuppressive consequences of testosterone (Folstad & Karter, 1992), and the shift of energetic resources from the immune system to activities associated with mate acquisition and reproduction. Spleen size appears to reflect variation in parasite load, whether in terms of parasite richness or intensity (Shutler *et al.*, 1999; Morand & Poulin, 2000; Cowan *et al.*, 2009; Schulte-Hostedde & Elsasser, 2011), and thus, because males tend to be more parasitized than females, males should have larger spleens than females. Our results contradict this prediction – in all cases for which sufficient data were available, females had relatively larger spleens than males. Similarly, among birds, females have larger spleens than males in a significant number of species (Møller, Sorci & Erritzoe, 1998b. but see Robinson *et al.*, 2008). In contrast, despite male-biased parasitism in masked shrews (*Sorex cinereus*) (Cowan *et al.*, 2007), males and females have similarly sized spleens (Cowan *et al.*, 2009). In grey squirrels (*Sciurus carolinensis*), no sex differences in spleen mass were observed (Scantlebury *et al.*, 2010). An alternative perspective is that because males are unable to invest heavily in the immune system, their spleens are smaller relative to females. Consistent differences in spleen size thus may reflect inherent differences in immune system investment between the sexes.

The energetic cost of immune function is the basis for the presumption that the immune system is a significant component of an individual's energy budget (Sheldon & Verhulst, 1996). The spleen, as an important component of the immune system, is thus expected to be costly to maintain, and thus condition-dependent (Møller *et al.*, 1998a; Schulte-Hostedde & Elsasser, 2011). In general, spleen mass was positively related to body condition among all groups of mink for which sufficient data were available. Regardless of the origin of the population, individuals in good condition had relatively larger spleens than those in poor condition. These patterns are similar to those found in other studies of American mink (Schulte-Hostedde & Elsasser, 2011), and other mammals and birds (Møller *et al.*, 1998a; Vicente *et al.*, 2007). Thus, in an environment in which energy is finite, individuals in good condition are expected to have higher levels of investment in the immune system (represented by the spleen) than those in poor condition. In a captive domestic context, however, energy is not expected to be limited, and thus condition dependence of the immune system (the spleen) is predicted to be lessened or absent. Domestic mink are maintained in individual pens with a suitable nest and bedding within sheds that protect them from environmental extremes [Fur Institute of Canada Fact Sheet – About mink Farming (accessed at http://www.fur.ca/files/info_sheets/about_mink_farming.pdf on 19 April 2012)]. Mink are fed daily (twice a day during periods of growth) with a diet that meets their nutritional needs and provided with water *ad libitum* [National Farm Animal Care Council of Canada – Recommended Code of Practice For the Care and Handling of Farm Animals – Mink (accessed at http://www.nfacc.ca/pdfs/codes/factsheets/Mink%20Fact sheet.pdf on 23 January 2012)]. These conditions would appear to be relatively benign from the energetic perspective of free-ranging mink, and so domestic mink are expected to be experiencing reduced energetic costs associated with thermoregulation, acquisition of food, etc. Our results tend to support this hypothesis – the average partial correlation coefficient of wild male mink was steeper than the average partial correlation coefficient of domestic male mink, although pair-wise comparisons showed no significant differences. Further study of this issue with a larger sample of populations would provide more concrete evidence with which to test this hypothesis.

We have concluded that differences in spleen mass between wild and domestic mink, and between feral domestic mink and domestic mink on farms are the result of differences in the prevalence and abundance of parasites and pathogens. Nonetheless, spleen size can be affected by other factors, including age (Ottinger & Lavoie, 2007; Cowan *et al.*, 2009), stress (McEwan *et al.*, 1997), and season (Silverin *et al.*, 1999). We argue that age is unlikely to be a factor because even if wild and domestic mink vary in age, age differences are unlikely to explain differences in spleen mass between domestic and feral mink, given that feral mink had escaped from mink farms. Stress levels experienced in a domestic and natural environment may differ, and so further investigations should determine cortisol levels of animals in both contexts.

Future work should use an experimental approach to further test the predictions outlined here. For example, the prediction that the veterinary care provided to domestic mink leads to reduced immune investment can be tested by conducting an experiment that involves medicating and vaccinating a wild population of mink. Furthermore, an unexamined issue associated with domestic mink is the hybrid mink that are produced when wild and domestic mink

produce offspring (Kidd *et al.*, 2009). Immune function of hybrid mink is unknown, and it would be of interest to examine how variation in parasite load and body condition affects immune investment. Finally, the use of alternative measures of immune investment should be explored (e.g. cellular assays, immune challenge protocols) (Boughton, Joop & Armitage, 2011) to test the effects of parasites and energy reserves on immune function in the wild–domestic mink system.

ACKNOWLEDGEMENTS

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