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Original study

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Long-term stress level in a small mammal species undergoing range expansion

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Abstract: Rapid climate change is currently altering species distribution ranges. Evaluating the long-term stress level in wild species undergoing range expansion may help better understanding how species cope with the changing environment. Here, we focused on the white-footed mouse (*Peromyscus leucopus*), a widespread small mammal species in North-America whose distribution range is rapidly shifting northward. We evaluated long-term stress level in several populations of *P. leucopus* in Quebec (Canada), from the northern edge of the species distribution to more core populations in Southern Quebec. We first tested the hypothesis that populations at the range margin are under higher stress than more established populations in the southern region of our study area. We then compared four measures of long-term stress level to evaluate the congruence between these commonly used methods. We did not detect any significant geographical trend in stress level across our study populations of *P. leucopus*. Most notably, we found no clear congruence between the four measures of stress level we used, and conclude that these four commonly used methods are not equivalent, thereby not comparable across studies.

Keywords: corticosterone; fluctuating asymmetry; *Peromyscus leucopus*; spleen; stress level.

1 Introduction

Climate warming is currently occurring at an unprecedented rate (IPCC 2014; Rahmstorf et al. 2007), and human activities are considered the strongest drivers of climate change (Ellis et al. 2013; Raftery et al. 2017). Over the last decades, global average temperature has drastically increased (Hansen et al. 2010; Ji et al. 2014), and the 20 warmest years in the 134-year record all have occurred since year 2001, with the exception of 1998. Furthermore, the year 2020 ranked as the warmest on record, tied with 2016 (NASA 2021). Species are responding to these rapid changes in the environment, many of them by altering their distribution range (Parmesan 2006; Root et al. 2003). However, species differ in their ability to cope with environmental changes (Hill et al. 2002), and there is ample evidence for local extinction (Pounds et al. 2006), range contraction (Flousek et al. 2015), expansion (Thomas and Lennon 1999) or range shift (Perry et al. 2005).

Stress level of an individual can vary greatly in response to habitat disturbance or environmental change (Bortolotti et al. 2009). Stress level in individuals within a population may also modulate the local distribution of a species through its effect on abundance, home range area, and dispersal rate (Gaitan and Millien 2016). Elevated stress level can lead to the reallocation of an individual's resources toward an activity that will enhance its short-term survival, such as foraging for feeding or escaping from predators, at the expense of allocating resources to territorial defense, reproduction or immunity (Sapolsky et al. 2000). However, such shift in resource allocation may decrease the fitness of the individual and its long-term survival (Charbonnel et al. 2008). For these reasons, stress level is commonly monitored in ecological or conservation studies (e.g. Davies et al. 2013; Rangel-Negrín et al. 2009; Sheriff et al. 2011; Suorsa et al. 2003). Two theories aim at explaining the variation in health status along a gradient from the core to the edge of a species distribution range. First, the central-marginal hypothesis states that species are increasingly maladapted to an environment that deteriorates from optimal conditions at the center of their

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distribution to the edges of their range (Brown 1984; Brown et al. 1995; Eckert et al. 2008; Sexton et al. 2009). Under the central-marginal hypothesis, suboptimal biotic or abiotic conditions in peripherical populations is associated with a reduced genetic diversity, a lower abundance, and poorer condition of individuals in populations at the edge of the species range (Brown et al. 1995; Sagarin and Gaines 2002). Such characteristics of peripherical populations are predicted to be the most marked in temperate species in which individuals occurring at higher latitude are under harsher climatic and habitat conditions, such as colder or longer winter or limited food resources due to a shorter growing season. Accordingly, populations at the edge of a species distribution are expected to display larger stress level compared with more central populations (Cornelissen and Stiling 2010; Kark 2001). Another theory, the enemy-release hypothesis, states that as a species is expanding from its historical range, it may not be necessarily followed by its co-evolved natural enemies such as parasites or predators, resulting in individuals in better health in marginal populations compared to core populations (Jeschke and Heger 2018; Keane and Crawley 2002; Torchin et al. 2002). As a result, marginal populations should display lower stress level than more central ones. While under the central-marginal hypothesis, the rate of range expansion is expected to be limited by environmental conditions, a lower burden of parasites in marginal populations may increase the ability of individuals at the most northern limit of the range of a species to disperse, and thus increase the rate of range expansion in this species. The two mechanisms may very well interact simultaneously upon marginal populations, and their relative strength is most likely context-dependent.

There are different methods for estimating stress level, most of them relying on the measurement of physiological (Sheriff et al. 2011) or morphological (Leary and Allendorf 1989) traits. The most commonly used methods allow the measurement of short-term stress level by evaluating changes in the activity of the hypothalamic–pituitary–adrenocortical system through the measurement of glucocorticoid or metabolite concentrations in plasma, saliva, urine, or faeces (Sheriff et al. 2011). In studies focusing on wildlife, the estimation of long-term stress level is generally preferred, as the capture and handling of the studied individual have no significant effect on long term stress level while it may affect short-term stress level (Ashley et al. 2011). An estimate of long-term stress level can be derived from the quantification of the main glucocorticoid hormone in rodents, the corticosterone, extracted from the hair matrix. This measure returns averaged stress levels underwent during the entire duration of the hair growing period (Meyer and Novak 2012). The adrenal gland mass is also reflecting long-term stress level, as chronic stress may induce hyperplasia in the adrenal glands (Ulrich-Lai et al. 2006). Finally, the level of fluctuating asymmetry in a bilateral skeletal structure has also been used to estimate the level of stress in an individual. Individuals living in a stressful environment may develop an asymmetry between the left and right sides of their anatomy during their development (Leary and Allendorf 1989; Parsons 1990) and this asymmetry can be quantified using morphometric methods (Klingenberg et al. 2002). To our knowledge, the comparison of fluctuating asymmetry levels with physiological measures of stress, such as corticosteroids or the adrenal gland mass has never been done.

The aim of our study was therefore to use three distinct methods of estimation of long-term stress level in a widespread species, the white-footed mouse (*Peromyscus leucopus*), currently undergoing rapid range expansion into Southern Quebec. We tested the hypothesis that stress level in this species is increasing towards the northern range limit of its range (the central-marginal hypothesis). We also evaluated the congruence between the three distinct methods we used to estimate stress level in *P. leucopus* (hair corticosterone level, adrenal gland mass and fluctuating asymmetry in skeletal structures), as a mean of evaluating their effectiveness and reliability as a measure of stress.

2 Materials and methods

2.1 Field sampling

We sampled twelve white-footed mouse populations between June and August in 2011, 2012, and 2013 across Southern Quebec, between 45.04 and 46.30°N latitude (Figure 1). At each sampling site, 160 Sherman (H. B. Sherman Traps, Inc, Tallahassee, FL) live traps were placed every 10 m in grids of 40 traps (i.e. four grids of four by 10 traps at each site). The traps were baited with a mixture of oat and peanut butter late afternoon and checked the following morning for three consecutive nights. *Peromyscus* individuals were identified to the species level using species-specific genetic markers as described in Rogic et al. (2013).

The populations of *P. leucopus* in Southern Quebec belong to three distinct post-glacial lineages that are today separated by water bodies André et al. 2017a; Fiset et al. 2015; Garcia-Elfring et al. 2017. The most northern lineage expanded from a central glacial refugium, and colonized the regions north of the St-Lawrence river. Two additional lineages are occurring on the southern side of the St Lawrence river, separated by the Richelieu river, and both these lineages expanded from a Southeastern glacial refugium. We thus assigned each of our populations to three distinct lineages (North, South-West

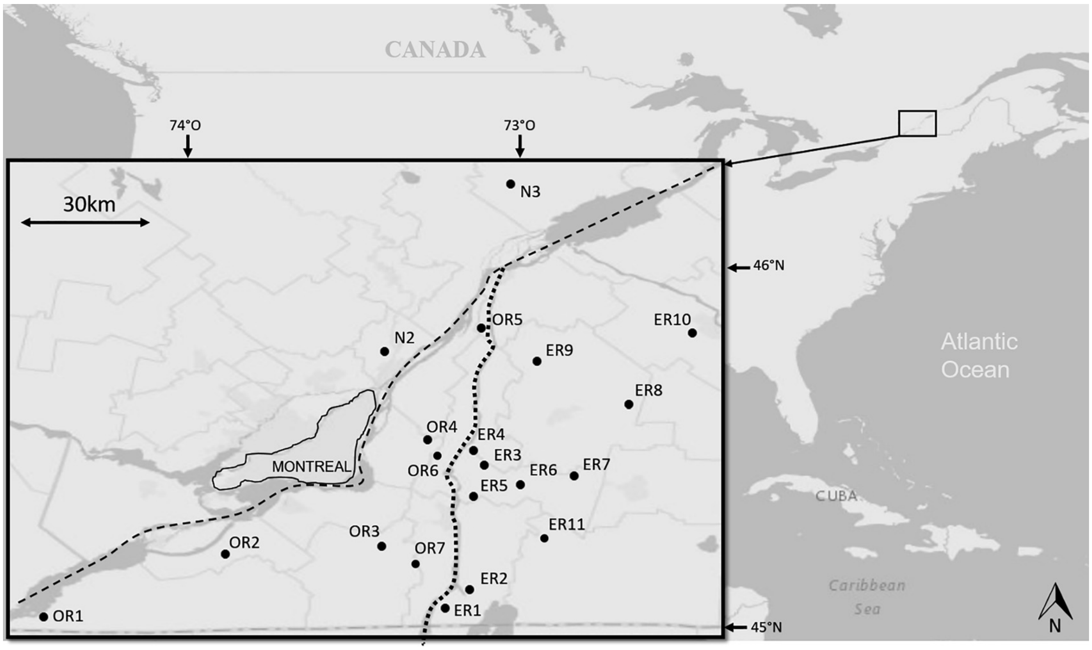

Figure 1: Location of the sampling sites (N2, N3: northern lineage; OR1, OR2, OR3, OR4, OR5, OR6, OR7: Southwestern lineage; ER1, ER2, ER3, ER4, ER5, ER6, ER7, ER8, ER9, ER10, ER11: Southeastern lineage). The St-Lawrence and Richelieu rivers are represented by the dashed and dotted lines respectively.

and South-East). These three lineages are currently expanding into Southern Quebec along a Southwestern-Northeastern direction (Roy-Dufresne et al. 2013; Simon et al. 2014). Populations ER10, south of the St Lawrence river, and N3, north of the St Lawrence, were considered to be at the species range margin as no *P. leucopus* individual was captured further north during our field surveys. We then calculated the shortest Euclidean distance from each site to the most Southern site in our study area within each lineage (Northern lineage: N1; Southwestern lineage: OR1; Southeastern lineage: ER1) using the package geosphere in R (Hijmans 2016). Populations located at the highest latitude or the most distant population from these three southern locations are thus the most peripheral, close to the range edge of the distribution of *P. leucopus* in southern Quebec.

2.2 Estimation of long-term stress level (LSTL)

2.2.1 Hair corticosterone: We measured the amount of corticosterone in hair samples from a subset of individuals trapped in 2013. The measurements were taken from 75 specimens from eight distinct populations across our study area (Table 1). Hairs were collected by fur-clipping the rump of each individual as close as possible to the skin. The surface of the sample was washed, since it may have been soiled with urine or faeces that also contain steroid hormones when the animal was in the trap. We used a protocol adapted from Macbeth et al. (2010) (protocol detailed in the supplementary material). Because no validation of the method has ever been performed for *P. leucopus*, a parallelism test between authentic corticosterone standards and serially diluted hair extract was performed to detect immunological similarities between the standard and sample hormones (Plikaytis et al. 1994).

Table 1: Study populations and sample size for the estimation of long-term stress level.

Site	Hair corticosterone	Adrenal gland mass	Skull FA score	Lower jaw FA score
ER1	10	20	36	32
ER2	8	8	13	13
ER3	0	0	27	25
ER4	0	0	25	22
ER5	0	0	18	14
ER6	8	7	28	25
ER7	0	0	19	14
ER8	0	0	14	13
ER9	0	0	7	7
ER10	11	11	30	29
ER11	0	4	7	7
N2	0	0	5	4
N3	10	15	27	26
OR1	0	4	11	11
OR2	10	12	19	19
OR3	7	15	15	13
OR4	10	9	10	10
OR5	0	0	4	4
OR6	0	5	5	5
OR7	0	5	5	5
Total	74	115	325	298

2.2.2 Mass of the adrenal glands: We then measured the mass of the adrenal glands, that are known to be larger in stressed individuals (Ulrich-Lai et al. 2006) and was found to be negatively related with the size of the home range and movement rate in *P. leucopus* (Gaitan and Millien 2016). The analyses were performed on 115 specimens from 11 different populations including the eight populations selected for the corticosterone assay. Adrenal glands were extracted from the trapped animals and conserved in ethanol. They were then dried at 90 °C for 24 h and weighed with a precision scale to the nearest 0.00001 g (Gaitan and Millien 2016).

2.2.3 Fluctuating asymmetry in the skull and lower jaw shapes: We measured the level of fluctuating asymmetry in the shape of the skull and of the lower jaw from our sampled individuals. The measurements were taken for 20 populations (including the 11 populations from the previous method), on a total of 325 and 298 individuals for the skull and the lower jaw, respectively (Table 1). Pictures of the ventral side of the skull (i.e. cranium) and the labial side from the left and right lower jaws (i.e. mandibles) were taken using a Lumenera Infinity one digital camera mounted on a Leica MS5 stereomicroscope coupled with a Leica ×0.32 Achromat lens. Two-dimensional landmarks were digitized using the TPS Dig software (Rohlf 2010). Three unpaired and 22 paired landmarks were located on the ventral cranial side (Supplementary Figure S1) and eight landmarks were located on the labial side of the right lower jaws and their corresponding mirrored left lower jaw image (Supplementary Figure S2). Measurement error was assessed by digitizing three times 25 and 42 samples randomly selected for skulls and lower jaws, respectively. Two separate Procrustes superimpositions were then performed for skulls and lower jaws using Morpho J software (Klingenberg 2011) followed by Procrustes ANOVAs to detect the effects of individual, directional asymmetry and fluctuating asymmetry in the skulls and lower jaw configurations (Klingenberg et al. 2002). We calculated FA scores of the skull and lower jaw shapes, that quantify the individual asymmetries of shape as deviations from the mean asymmetry in the sample. Individual FA scores were computed with the MorphoJ software, using the Procrustes distance, which corresponds to a measure of absolute shape differences and treats shape deviations from the sample mean equally, regardless of their direction (Dryden and Mardia 1998). The data was checked for the presence of allometry (Palmer and Strobeck 2003), the symmetric variation due to variation in size, by regressing individual FA scores on centroid size.

2.3 Statistical analyses

Prior to all analyses, we applied a square root transformation to the cortisol level and adrenal gland mass variables, as well as a logarithmic transformation to both fluctuating asymmetry variables (skull and lower jaw). We also calculated the residuals of the adrenal gland mass against body mass as in Gaitan and Millien (2016), and used these residuals as a response variable in our models. As body mass was correlated with sex ($F = 21.93$, $p < 0.001$), we opted to include only the Sex variable in our models to avoid collinearity in the explanatory variables. However, both distance from the most southern site within each lineage and latitude were include in the models, since these two variables were not correlated ($r = -0.02$, $p = 0.69$).

2.3.1 Sexual dimorphism and congruence between the four LSTL evaluation methods: We first tested for sexual dimorphism in the four stress variables we used to estimate long-term stress level (hair cortisol, adrenal gland mass and fluctuating asymmetry in the skull and the lower jaw) with a mean comparison *t*-test using the stats package in R (R Core Team 2017). To evaluate the congruence between the four distinct stress variables, we then performed multiple pairwise Pearson correlation tests adjusted with a Bonferroni correction using the package psych in R (Revelle 2017).

2.3.2 Geographical gradients in stress levels: We tested for a difference in long term stress level across sites with linear models for each stress level variable and included the variables Site and Sex as independent variables. We further tested the hypothesis that long-term stress level increased towards the range edge of *P. leucopus* with linear models, by including in the models the variables Latitude, Distance to the most southern site (within each lineage), and Sex as explanatory variables. We ran distinct models for each stress variable separately.

3 Results

3.1 Long-term stress level estimates

3.1.1 Hair corticosterone quantification

Intra and inter assay coefficients of variation were respectively 13.95% ($n = 108$) and 12.48% ($n = 5$). Results from serially diluted extracts of hair were parallel ($r^2 = 0.95$, $p < 0.001$) with results from serially diluted cortisol standards provided with the EIA kit (Supplementary Figure S3). This latter result confirms the validation of the method used here. The corticosterone concentrations in hair ranged from 0.6 to 44.8 pg/mg of hair with a mean of 13.7 ± 1.3 pg/mg (Table 2).

3.1.2 Adrenal gland mass

AGM ranged from 0.25 to 2.65 mg with a mean of 1.241 mg ± 0.04 mg (Gaitan and Millien 2016; Table 2).

Table 2: Summary statistics for the four long-term stress level variables.

	n	Mean	SE	Min	Max
HC	74	13.8	1.3	0.6	44.8
AGM	115	1.26	0.04	0.25	2.65
SFA	325	0.0071	0.0001	0.0035	0.0168
LJFA	298	0.0207	0.0005	0.0082	0.0609

HC, hair corticosterone (pg/mg); AGM, adrenal gland mass (mg); SFA, skull fluctuating asymmetry; LJFA, lower jaw fluctuating asymmetry; *n*, sample size; Mean, mean value; SE, standard error of the mean; Min, minimum; Max, maximum.

3.1.3 Fluctuating asymmetry

The measurement error for skull and lower jaws were estimated and mean squares of FA, DA, and individual variation were found to exceed the error component, indicating that the contribution of measurement error to overall shape variation was not significant. Procrustes ANOVAs were performed separately for skull and lower jaw data. For each skeletal structure, we found significant FA (Individual × Side interaction), DA (Side effect), and differences among individuals. Skull Procrustes fluctuating asymmetry scores ranged from 0.0035 to 0.0168 with a mean of 0.0071 ± 0.00012. Lower jaw Procrustes fluctuating asymmetry scores ranged from 0.0082 to 0.0609 with a mean of 0.0207 ± 0.0005 (Table 2). No significant allometric effect was detected ($p > 0.05$).

3.2 Sexual dimorphism and congruence between the three long-term stress level measures

Sexual dimorphism was apparent in two of our stress variables: males had a larger adrenal gland mass than females ($t = -3.71$, $p < 0.00052$), while females tended to have a higher cortisol level than males ($t = 3.13$, $p < 0.004$). We did not detect any significant sexual dimorphism in FA scores for skulls ($p = 0.26$) or lower jaws ($p = 0.06$). Furthermore, there was no significant correlation between the individual stress level values obtained from the three distinct LTSL evaluation methods we used (all $p > 0.05$, probability values adjusted for multiple tests) (Figure 2).

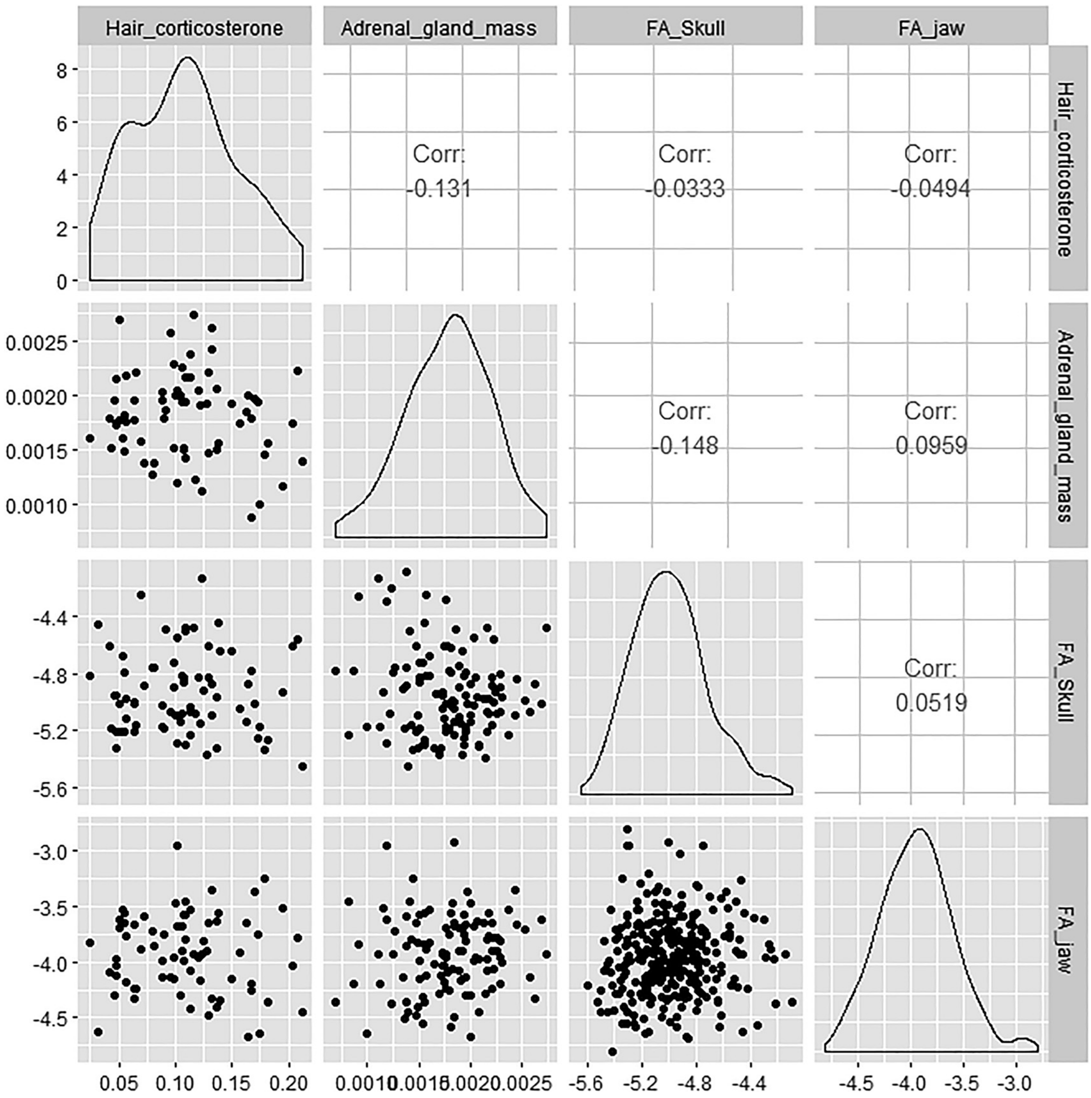

Figure 2: Scatterplot matrix of the four long-term stress level variables. Pearson R correlation coefficients between each pair of variables are indicated on the upper part of the matrix, with significance levels adjusted for multiple comparisons.

3.3 Geographical variation in long term stress level

The *P. leucopus* populations in our study did not present any significant differences between sites in stress level estimated from the two fluctuating asymmetry indices or from the cortisal level (Table 3). In contrast, the mass of the adrenal gland varied across sites in our study area (Table 3). There was no significant effect of the distance to the most southern site within each lineage or of latitude on three of the stress variables we used, namely, cortisol level and the two FA scores (Table 4). Conversely, there was a strong significant effect of latitude on the mass of the adrenal gland. Finally, latitude had an effect on the FA score of the skull, but only when interacting with sex.

4 Discussion

4.1 Congruence between the three LSTL evaluation methods

One objective of our study was to compare individual long-term stress level estimates obtained using three distinct methods. Interestingly, we did not detect any correlation between these three distinct measures of stress. Furthermore, we observed opposing sexual dimorphism between the hair corticosterone level and the adrenal gland mass. These results are much surprising, as we should expect a correlation between variables that are all known to estimate long-term stress level. One might argue that these methods do not actually measure the same traits. Fluctuating asymmetry, for example, is the result of skeletal development imperfections caused by some stress during the development of an individual (Leary and Allendorf 1989; Parsons 1990), whereas hair cortisol and adrenal gland mass are representative of stressors experienced by the same individual over the few weeks preceding its capture (Mastromonaco et al. 2014; Ulrich-Lai et al. 2006). As a result, one individual could have been born to a mother subjected to significant stressors during gestation, impacting the development of embryos and leading to developmental instability, estimated here by fluctuating asymmetry in skeletal traits. However, hair corticosterone level and the adrenal gland mass measure the level of stress experienced by this same individual, preceding its capture. Different stress levels are therefore measured, depending on the method used. Such pattern would be apparent for example in animals born during a period of the year characterized by harsher conditions, inducing a high degree of fluctuating asymmetry in their morphology, but who would be captured in summer, when environmental conditions are more favorable, generating lower corticosterone concentrations in their hairs. Furthermore, the effectiveness of fluctuating asymmetry as a proxy for stress level during development has been questioned (Bjorksten et al. 2000). Leung and Forbes (1996) argued that FA measures are rather species-specific, stressor-specific, and trait-specific. Our results corroborate this hypothesis, as we found that our two measures of FA in two functionally connected skeletal structures within a single species were not correlated. Similarly to our finding, the literature abounds with publications (Brakefield 1997; Campbell et al. 1998; Roy and Stanton 1999; Woods et al. 1999) in which FA correlates with stress for a particular trait but not for others, even if these traits are functionally linked. Similarly, Zachos et al. (2007) reported a lack of a

Table 3: ANOVAs of the linear models testing for the effect of site, and sex on each of the four stress variables.

		Sum sq	Mean sq	NumDF	*F* value	Pr (>*F*)
HC	Site	0.0115	0.0016	7	0.8993	0.5123
	Sex	0.0235	0.0235	1	12.8748	**0.0006**
	Residuals	0.1224	0.0018	67		
AGM	Site	1.7066E-06	5.1209E-07	11	2.8123	**0.0030**
	Sex	1.7066E-06	1.7066E-06	1	9.3726	**0.0028**
	Residuals	1.9301E-05	1.8209E-07	106		
SFA	Site	0.7590	0.0399	19	0.4605	0.9757
	Sex	0.1205	0.1205	1	1.3887	0.2395
	Residuals	27.0665	0.0868	312		
LJFA	Site	1.3980	0.0736	19	0.5897	0.9128
	Sex	0.3240	0.3243	1	2.5987	0.1081
	Residuals	35.3180	0.1248	283		

The models were simplified to remove the interactions terms that were not significant in the full models. Bold face values indicate statistical significance.

Table 4: ANOVAs of the linear models testing for the effect of latitude, distance to the most southern site within each lineage, and sex on the four variables we used to estimate long-term stress levels.

		Sum sq	Mean sq	Df	*F* value	Pr (>*F*)
HC	Latitude	0.0001	0.0001	1	0.0659	0.7982
	Distance	0.0060	0.0060	1	3.2817	0.0742
	Sex	0.0200	0.0200	1	10.9641	**0.0015**
	Residuals	0.1313	0.0018	72		
AGM	Latitude	2.8752E-06	2.8752E-06	1	15.6979	**0.0001**
	Distance	7.9300E-08	7.9300E-08	1	0.4331	0.5118
	Sex	2.6230E-06	2.6230E-06	1	14.3206	**0.0002**
	Residuals	2.1063E-05	1.8316E-07	115		
SFA	Latitude	0.0760	0.0760	1	0.9140	0.3398
	Distance	0.1492	0.1492	1	1.7937	0.1814
	Sex	0.0872	0.0872	1	1.0488	0.3065
	Latitude: sex	0.3576	0.3576	1	4.3008	**0.0389**
	Residuals	27.2758	0.0832	328		
LJFA	Latitude	0.1920	0.1920	1	1.5846	0.2091
	Distance	0.1340	0.1340	1	1.1007	0.2950
	Sex	0.2890	0.2890	1	2.3835	0.1237
	Residuals	36.4260	0.1214	300		

The models were simplified to remove the interactions terms that were not significant in the full models. Bold face values indicate statistical significance.

consistent correlation between metric and non-metric FA measures in roe deer, and Keller et al. (2007) detected a significant increase in FA of the molar shape, but not in FA of the molar size in mice subjected to a dioxin based toxicant. Finally, FA measures were originally meant to quantify stress at the population level rather than at the individual level (Knierim et al. 2007), questioning the relevance of studying within-population variation in FA. Stress evaluation methods based on glucocorticoid quantification may be more reliable, as they have been through more validation studies under controlled conditions (Charmandari et al. 2005; Cook 2012; Dickerson and Kemeny 2004; Romero 2004). Unfortunately, these validations have not been applied yet to hair corticosterone, which remains a relatively recent technique. Validation studies should also be conducted for the method based on the adrenal gland mass as it might constitute a promising and affordable method to estimate individual stress levels. Our results thus confirm the need for caution when evaluating stress level in wildlife populations, and interpretating results on variation in the estimated stress levels.

4.2 Spatial heterogeneity in the long-term stress level

The central marginal hypothesis states that individuals are increasingly maladapted to an environment which deteriorates from the core to the edges of their distribution range (Brown 1984; Brown et al. 1995; Eckert et al. 2008; Sexton et al. 2009). Here, we tested this hypothesis using three distinct methods of stress level estimation in the widespread white-footed mouse (*P. leucopus*), and two measures of the degree of periphery of these populations. We did not detect any significant effect of the distance to the most southern site within each lineage for any of the stress level measures we considered. Latitude did not have any effect on stress level either, except when considering the mass of the adrenal gland as a measure of stress.

We attribute such overall negative result to both our study design and the biology of our study species. First, our sample sizes were relatively low for some of the stress measures we used, ranging from 7 (cortisol), 11 (adrenal gland) to 19 (FA) populations. Furthermore, our study area covered a south-west/north-east gradient of approximately 200 km long, which may not be large enough to capture subtle variation in stress level. Previous studies on variation in stress level in response to environmental stressors covered a much larger area than our study area. Liebl and Martin (2012, 2013) studied the invasion of an introduced songbird on an 885 km gradient and detected a higher corticosterone level in peripheral populations. Similarly, Kark (2001) and Cornelissen and Stiling (2010), who worked respectively on *Alectoris chukar* on a 322 km gradient and on two *Quercus* species on an 850 km gradient, found more asymmetric specimens in peripheral populations compared to central ones. Yet, Siikamäki and Lammi (1998) found more asymmetric plant specimens of *Lychnis viscaria* in marginal populations, on a studied range as short as 150 km. The white-footed mouse occurs across much of the eastern side of the north American continent, down from Mexico up to Southern Canada. Up until only a few decades ago. The white-footed mouse was relatively rare in Southern Quebec, and small mammal communities were dominated by the endemic congeneric species, *Peromyscus maniculatus* (Millien et al. 2017; Roy-Dufresne et al. 2013). One may thus argue that all individuals occurring in Southern Quebec where populations of *P. leucopus* are still expanding, are all experiencing a relatively large stress, when compared to individuals closer to the core of the distribution range of this widespread species. Increasing the breadth of our sampling area may thus have allowed to capture a significant geographical variation in stress level across populations of *P. leucopus*.

Alternatively, the lack of variation in stress level in *P. leucopus* within our study area may also be the result of a high adaptive potential in the white-footed mouse, with recently established populations already well adapted to their novel environment. Given its generalist behavior (Lackey et al. 1985) and current rapid rate of expansion into southern Quebec (Roy-Dufresne et al. 2013), it is reasonable to expect the white-footed mouse to possess a high adaptive potential. It has been shown that *P. leucopus* populations from southern Quebec are currently rapidly evolving, likely in response to novel environmental conditions during range expansion (Garcia-Elfring et al. 2017, 2019; Millien et al. 2017), supporting the hypothesis that *P. leucopus* has the potential to adapt rapidly to changing environments. This last hypothesis is in line with niche models predicting the future expansion of the white-footed mouse into Quebec under climate warming at unprecedented rates (André et al. 2017b; Roy-Dufresne et al. 2013; Simon et al. 2014). This is especially concerning for Public Health in the region, as the white-footed mouse is the main reservoir species for the pathogen causing Lyme disease, *Borrelia burgdorferi*. The rapid geographical range shift and expansion into southern Quebec of the white-footed mouse, a reservoir species which is reputably asymptomous when carrying *B. burgderferi* (Schwanz et al. 2011) and very competent for this pathogen transmission (Donahue et al. 1987), combined with an increase in abundance and an apparent lack of additional stress in expanding populations all are critical factors likely favouring the rapid emergence of Lyme disease in the region.

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Research ethics: All procedures were approved by the Ministère des Ressources Naturelles et de la Faune du Québec (SEG Permit #2011-05-15-014-00-S-F SEG, #2012-07-16-1417-16-17-SF and #2013-07-04-14-16-17-SF), and McGill University Animal Care Committee (AUP#5420).

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