Comparison of reproductive investment in native and non-native populations of common wall lizards reveals sex differences in adaptive potential

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Non-native animals can encounter very different environments than those they are adapted to. Functional changes in morphology, physiology and life-history following introduction show that organisms can adapt both fast and efficiently. It remains unclear, however, if female reproductive characters and male sexually selected behaviour show the same adaptive potential. Furthermore, the invasion success and evolutionary trajectory of non-native species might often depend on the ability of the sexes to coordinate shifts in characters associated with reproductive strategy. The common wall lizard, *Podarcis muralis*, has been repeatedly introduced from southern Europe to England over the past 80 years. Lizards in England experience a cool, seasonal climate that effectively restricts recruitment to the first clutch of the season, whereas in their native range up to three clutches per season recruit. As a consequence, both females and males in non-native populations should benefit from reducing or even eliminating their reproductive investment in second clutches. Using a combination of field data and experiments, we show that non-native females produce relatively larger and heavier first seasonal clutches and smaller and lighter second seasonal clutches compared to native females. In contrast, non-native and native males do not differ in their territorial and sexual behaviour later in the season. An adaptive shift in male seasonal reproductive investment may be constrained because males use breeding females as cues for sexual behaviour. If this is so, we expect a general pattern across climatic regimes whereby female reproductive investment evolves first, with responses in males lagging behind.

Introduced species are outstanding models to study phenotypic evolution (Reznick and Ghalambor 2001, Prentis et al. 2008). New abiotic and biotic conditions can abruptly change selective regimes and cause rapid shifts in morphology, physiology and life-history (Blossey and Notzold 1995, Huey et al. 2000, Yeh and Price 2004). Phenotypic variation along environmental clines typically involves both plasticity and genetic divergence (Robinson and Wilson 1996, Hoffmann et al. 2005, Buckley et al. 2010), and the same is true for differences between native and non-native populations (Lee et al. 2003). Although it is often difficult to know if the observed shifts in non-native populations are in the direction favoured by selection, this inference is strengthened when the adaptive value of phenotypic clines in the ancestral range is well established (Losos et al. 1997, Gilchrist et al. 2001, Hoffmann et al. 2002).

Reproductive life-history traits (e.g. relative investment, timing, frequency and duration of reproductive events) often vary adaptively within and between species with changes in temperature and seasonality, along latitudinal and altitudinal gradients (Niewiarowski 1994, Rose and Lyon 2013, Du et al. 2014). For example, in multi-clutching ectotherms, females in populations at high latitudes typically invest relatively more in the first seasonal reproductive event (Forsman and Shine 1995, Roig et al. 2000), sometimes resulting in the production of a single clutch per year in a cool climate and several clutches in a warm climate (Pincheira-Donoso and Hunt 2015, also see Bestion et al. 2015). Therefore, females introduced to a comparably cooler climate should exhibit a similar seasonal shift in their reproductive allocation.

Climatic effects on the timing, frequency, duration or success of female reproductive events should also cause concomitant variation in the adaptive value of male investment in reproduction across the breeding season. Thus, males should modify their sexual behaviour in accordance with expected fitness returns on investment (Hirshfield and Tinkle 1975). Indeed, numerous experimental studies have shown that males adjust their competitive behaviour and courtship effort based on the prevailing reproductive environment (Grant et al. 1995, Shine et al. 2003, Svensson et al. 2010), including in response to female reproductive potential (Jones et al. 2001, Reading and Backwell 2007).

Furthermore, sexual selection regimes can also change in response to climatic effects on the availability of mating opportunities (Emlen and Oring 1977, Shuster and Wade 2003), with potential implications for the relative costs and benefits of male sexually selected traits. For example, variance in the ratio of receptive females to males (the operational sex ratio) between reproductive episodes could generate seasonal variation in the intensity and direction of sexual selection (Reichard et al. 2008, Wacker et al. 2014). If females become more synchronous in their receptivity, as is predicted in more seasonal environments (Ramírez-Pinilla et al. 2009), dominant males will be less able to monopolise multiple females, reducing the opportunity for sexual selection (Grant et al. 1995, Mendoza-Cuenca and Macias-Ordonez 2009). Thus, in response to a new climatic regime, the expression of male sexually selected traits could also shift in non-native populations, however, the direction of change, if any, is not easy to predict.

These considerations suggest that female reproductive life-history and male reproductive behaviour should shift concurrently following introduction to a different climatic regime, which could also result in changes to sexually selected traits. However, there is limited evidence that this is the case, and it is possible that female and male reproductive characters do not have similar adaptive potential. Here we take advantage of a series of introductions of common wall lizards *Podarcis muralis* from Italy into England, where the spring and summer temperatures are substantially lower. Extant populations in England are well characterized genetically and most originate from north-central Italy (approximately Tuscany and Bologna–Modena, Michaelides et al. 2015). In Italy, where the species is native, females have up to three clutches per breeding season. However, climatic conditions in England place significant restrictions on embryo development, resulting in highly reduced recruitment from second clutches (While et al. 2015a). Thus, female and male fitness is almost entirely dependent on their reproductive success in the first clutch of the season, with second clutches contributing little to the total number of surviving offspring produced. As a consequence, in non-native females we expect investment in second clutches to be reduced in favour of first clutches relative to native females. Further, we expect non-native males to invest less in their reproductive behaviour towards second clutches compared to native males. If reproductive responses in females have consequences for sexual selection regimes, this could also result in adaptive divergence between native and non-native populations in male sexually selected traits. We tested these predictions using a combination of field data and experiments in outdoor enclosures. Specifically, we 1) tested for divergence in female reproductive investment and male sexual characters (e.g. body size, head size, bite force, colouration), and the degree and direction of sexual dimorphism between native and non-native populations, 2) examined differences in patterns of reproductive investment in first and second clutches between females from the native and non-native range under standardized conditions, and 3) explored in experimental populations whether any shifts in female reproductive investment were accompanied by differences between native and non-native males in the intensity of male–male competition and courtship effort for second clutches.

Material and methods

Study populations

The common wall lizard, *Podarcis muralis*, is a small diurnal lacertid native to southern Europe. The species has established non-native populations within Europe and North America over the last century, primarily through the pet trade and deliberate introductions (Deichsel and Gist 2001, Schulte 2008, Schulte et al. 2012, Michaelides et al. 2015). From 2010 to 2015, 478 native (females (n = 196), males (n = 282)) and 655 non-native (females (n = 372), males (n = 283)) adult lizards (≥ 45 mm snout–vent length (SVL)) were captured from ten non-native populations in the south of England and eighteen native populations in northern Italy (Supplementary material Appendix 2 Table A2). These native and non-native localities differ substantially in their thermal environment with mean monthly maximum air temperatures during the main activity season for populations in England approximately 5–10°C lower than their source regions in northern Italy (While et al. 2015a). The first records of individuals at the non-native localities ranges from 1930 to 2004, and the genetic origin of the non-native populations sampled for the enclosures experiment can be traced to at least three sources in the native range (Michaelides et al. 2015). All populations included in our study comprise of lizards with a green-backed morphology that have pure Italian (Tuscan and/or Venetian) ancestry (Michaelides et al. 2015).

Morphological divergence

We captured all lizards at the start of the breeding season (March–April) to ensure that they were within their first seasonal reproductive episode. Abdominal palpation confirmed the presence of eggs in all females in this study unless otherwise stated. Upon capture, we recorded four morphometric measurements from each lizard: SVL, measured to the nearest mm with a ruler, body mass measured to the nearest 0.01 g using a digital scale, and head length and head width recorded to the nearest 0.1 mm using a dial caliper. In addition, two authors (GMW and TU) scored dorsal green colouration (greenness), based on an intensity scale from 1 to 10 following While et al. (2015b). One author (GMW) photographed all individuals on their ventral and left lateral side using a digital camera with white balance customised prior to each photo session to adjust for the background illumination (Wang and Shaffer 2008). From these photographs, we estimated ventral blackness (blackness) from the chest section (the region from the collar to the forelimbs) and outer ventral scale blue spot area (OVS blue area) from lateral images using the program ImageJ (<http://imagej.nih.gov>). For a sample of native and non-native lizards that were returned to laboratory facilities at Oxford University in 2013 and 2014, we also recorded maximum bite force (bite force, n = 122), and quantified outer ventral scale UV chroma (OVS UV chroma, n = 94) and hue (OVS hue, n = 94) from the reflectance spectra of males. Expanded details on the quantification of traits are given in Supplementary material Appendix 1, and Appendix 3 Table A3 gives a breakdown of sample sizes by trait, origin and sex.

Female reproductive investment

A sample of females (n = 303) collected between 2010 and 2015 that were carrying their first clutch of the season were returned to the laboratory. We housed the females individually within plastic terraria (590 × 390 × 415 mm) that contained sand substrate, a basking block, a shelter, and moist sand for egg laying. We kept the females under a 12:12 light/dark cycle. A 60 W spotlight above each cage provided opportunities for thermoregulation and UV light was provided with EXO-TERRAT 10.0 UVB fluorescent tubes. We fed the lizards daily (either two mealworms or two crickets) and sprayed the cages with water every second day. During this time, we checked the cages at least twice daily (a.m. and p.m.) for eggs. To quantify female reproductive investment, we retrieved and counted the number of eggs within each clutch, and noted the presence and number of infertile eggs (following Olsson and Shine 1997a). In addition, we measured clutch mass (fertile eggs only) and post-parturition body mass to the nearest 0.01 g using a digital balance. We housed 72 females collected in 2014 (native (n = 40), non-native (n = 32)) in experimental enclosures during their second seasonal receptive phase.

Male reproductive investment and sexual selection

Outdoor enclosure experiment

Lizards captured in 2014 from five native populations (80 lizards) and four non-native populations (64 lizards) were used in an enclosure experiment to compare reproductive investment towards second clutches in native and non-native lizards (see Supplementary material Appendix 2 Table A2 for population details). We obtained tissue samples from all individuals for genetic analyses by removing the tip of the tail, and preserved these in 90% ethanol. We confirmed the presence of eggs by palpating each female. In five cases the female had recently laid their first seasonal clutch prior to capture. We kept these females cool (4°C) to delay ovulation. All other lizards were housed individually as above until they oviposited. We kept most females for two days under lab conditions post-oviposition before their inclusion in the enclosure experiment. However, to enable the simultaneous release of receptive females into the enclosures, we kept some females at 4°C for additional days following oviposition to avoid progression through the next ovulation cycle.

We assigned each lizard to one of nine (~7 × 7 m) outdoor enclosures at the John Krebs Field Station, Univ. of Oxford, where the climate falls within the variation in the non-native range of wall lizards in England (While et al. 2015a; temperature loggers recorded mean daily temperatures in our enclosures that ranged from 11.6 to 22.6°C during the experiment). Each enclosure housed sixteen lizards of either native or non-native origin (8 male, 8 female). This is within the range of densities found under natural conditions (While and Uller unpubl.). Within every enclosure, individuals came from at least four populations with a minimum of three populations represented within each sex (see Supplementary material Appendix 1 for further details on assignment). Prior to release, we marked all lizards for identification at a distance with a unique number on their dorsal side using a non-toxic, non-hypoallergenic marker pen. Males were released into the enclosures a minimum of six days prior to the release of females to enable them to establish territories. Lizards of each sex were released within an enclosure simultaneously except in three cases where a single female was released within three days of the initial release of all the other females. At completion of the experiment, we recaptured and returned the lizards to the laboratory and housed them under standardized conditions. Five males (two native and three non-native) and one female (native origin) were not recaptured and presumed dead. Four non-native females did not produce a second seasonal clutch of eggs. For the remaining females (native (n = 39) and non-native (n = 28)) we recorded investment in second clutches, and took tail tissue samples from all second clutch juveniles to be used for the assignment of offspring paternity.

Collection of behavioural data

To quantify male investment in territory establishment and courtship, one author (HEAM) systematically observed the enclosures from the initial release of males until we confirmed that the females were in the late stages of gestation. During this time (~4 weeks) the observer recorded behavioural interactions within each enclosure population during 45 min observation periods. To reduce observer effects on behaviour, the observer was positioned outside the perimeter of the enclosure and movement was minimised during each observation period by using binoculars to scan the enclosure and identify lizards by their dorsal number tag. The identity of interacting lizards, the initial location of the receiver, and the nature of the social interaction were recorded according to an ethogram following Heathcote et al. (2016) (Supplementary material Appendix 12 Table A12).

From the observations, we categorised dyadic male-male territorial interactions (n = 414, the identity of both males was known in 395) and courtships (n = 511, mating was observed in 92) (further details provided in Supplementary material Appendix 1). For each male we quantified: total number of competitive interactions; relative number of courtships; relative number of females courted; relative mating success and relative fertilization success. The latter four measures were relative to the average of all males of the same enclosure, as this is the relevant comparison, and mating and fertilization success were based on paternity data (see details on paternity assignment below).

We calculated Dij-based David's dominance scores for each male within an enclosure (hereafter Dominance) based on the outcome of observed dyadic male–male territorial interactions. Dominance was calculated in R package 'Steepness' (de Vries 2011) following Gammell et al. (2003) with correction (to control for differences in the numbers of interactions between dyads) and normalisation (to control for the loss of males from three enclosures) described by de Vries et al. (2006) (see Supplementary material Appendix 1 for further details).

Paternity assignment

Following the experiment, native females produced 211 offspring (from 39 females in five enclosures), and non-native females produced 145 offspring (from 27 females in four

enclosures) from their second clutches. We isolated DNA from offspring and adult tissue samples following QIAGEN DNeasy extraction protocol in a final elution volume of 150 μl (in AE buffer). We carried out PCR reactions for 16 microsatellite markers with primers combined into five multiplexes (Richard et al. 2012, Heathcote et al. 2015, also see Supplementary material Appendix 5 Table A5), and assigned paternity using Cervus ver. 3.0 (Marshall et al. 1998), based on the trio (mother, father, offspring) LOD score using a strict confidence level of 95%. Offspring with more than one mismatching allele (21 native offspring) among mother–offspring–father trios and that amplified at fewer than three loci (one native offspring) were excluded from further analyses.

Strength and opportunity for sexual selection on males

To characterize and compare sexual selection on native and non-native males during the second within-season reproductive episode, we used a multiple index approach based on variance in mating and fertilization success (Jones 2009, Henshaw et al. 2016). For males of each origin, we estimated: 1) the Bateman gradient (β_{ss}), the slope of the least squares regression of relative mating success on relative fertilization (Jones 2009), 2) opportunity for overall selection (I), the variance in absolute fertilization success over the square of the mean fertilization success (Crow 1958), 3) the opportunity for sexual selection (I_S), the variance in absolute mating partners over the square of the mean total mating partners (Wade and Arnold 1980), and 4) the maximum standardized selection differential or Jones Index (S'_{max}), the product of β_{ss} and the square root of I_s (Jones 2009).

Statistical analyses

All statistical analyses were conducted in R ver. 3.1.2 (<www.r-project.org>) unless otherwise stated. For analyses with Gaussian distributed response variables we ran linear mixed models (LMMs) and for analyses with Poisson distributed response variables we ran generalised linear mixed models (GLMMs). For LMMs and GLMMs the significance of fixed effects are reported based on type III F-tests (with Kenward–Roger's approximation) and likelihood-ratio tests, respectively. All mixed model analyses of female investment included Population nested within Origin as a random effect. Enclosure was included as a random effect in mixed model analyses of male behaviour and sexual selection. For models with a significant interaction term including Origin (native or non-native), we performed post hoc tests to identify the sources of variation (implemented in R package 'multcomp', Hothorn et al. 2008), and report p-values that are adjusted for multiple comparisons.

Tests for divergence in morphology

To test for divergence in morphology and sexual dimorphism between native and non-native lizards we ran LMMs with each morphological trait as a response variable and Sex, Origin and a Sex by Origin interaction as fixed effects, and SVL as a covariate (where applicable). Because Head length and Head width were highly correlated (r = 0.81), we excluded Head width from analyses of divergence.

Tests for differences in female investment

We tested for differences in female reproductive investment (Clutch size, Clutch mass and Mean egg mass) in first clutches with a Poisson GLMM (for Clutch size) and LMMs (for Clutch mass and Mean egg mass) taking Origin, Post-parturition body mass and Year as fixed effects. To test for differences between native and non-native experimental females in their reproductive allocation between first and second clutches we ran LMMs for all three measures of investment with Origin, Clutch for female (first or second), their interaction, and Post-parturition body mass as fixed effects, and Female ID nested within Population and Origin as a random effect.

Tests for differences in male sexual behaviour

The 'steepness' of linear dominance hierarchies was proposed by de Vries et al. (2006) as a measure of the extent of asymmetry in dominance between adjacently ranked individuals whereby steep dominance hierarchies may reflect more intense social competition (Flack and de Waal 2004). To test for differences between the native and non-native experimental populations in the steepness of male dominance hierarchies, we ran a LMM with Dominance as the response variable, and Origin, Dominance rank (where 1 is the most dominant and 8 the least dominant male within an enclosure), and their interaction as fixed effects. To examine whether associations between territoriality and sexual behaviour differed between native and non-native males, we performed LMMs with relative number of courtships, relative number of females courted, and relative mating success as response variables. All models included Dominance (standardized: mean = 0, SD = 1), Origin and their interaction as a fixed effects, and male SVL (standardized: mean = 0, SD = 1) as a covariate.

Since body size is thought to be under strong sexual selection in wall lizards (Sacchi et al. 2009), and we predicted a relaxation in sexual behaviour for non-native males, we tested for Origin differences in the extent to which body size predicted male territorial and sexual behaviour. To generate an overall measure of male body size, we collapsed SVL, Head length, Head width and Body mass into a single principle component (Body size, Supplementary material Appendix A4 Table A4). For number of competitive interactions, we performed a Poisson GLMM with Body size, Origin and their interaction as fixed effects. For relative number of courtships, relative number of females courted, and relative mating success we performed LMMs with Body size (standardized: mean = 0, SD = 1), Origin and their interaction as fixed effects.

We used Mantel permutation tests (10 000 iterations) implemented in SocProg 2.4 (Whitehead 2009) to establish whether male investment in courting females predicted patterns of paternity i.e. as a possible indicator of post-copulatory processes (Olsson and Madsen 1998). Tie strengths for each male–female dyad were defined as absolute number of courtships and total number of offspring sired, for courtship and genetic networks, respectively. The p-values for native and non-native enclosures were combined using Fisher's method (Fisher 1932).

Comparison of the opportunity and strength of sexual selection on males

To compare the Bateman gradients of native and non-native males we ran a LMM with relative fertilization success as the response variable taking relative mating success, Origin and an interaction between relative mating success and Origin as fixed effects. To compare the opportunity for selection on native and non-native males we performed a Levene's test (Levene 1960) and a modified Levene's test (Brown and Forsythe 1974) on variance in male mating success (normally distributed) and variance in male fertilization success (non-normally distributed), respectively.

We tested for differences between native and non-native males in the associations between sexual traits and relative fertilization success since this could indicate shifts in male mating effort. Each trait was included as a fixed effect in a model with male Origin and a trait by Origin interaction, and relative fertilization success as the response variable. Traits were standardized (mean = 0, SD = 1) prior to analysis and SVL was included as a covariate where appropriate.

To compare the levels of multiple paternity in the native and non-native enclosure populations we ran a Poisson GLMM with number of fathers per clutch as the response variable, Origin as a fixed effect, and Clutch size as a covariate.

Data deposition

Data available from the Dryad Digital Repository: < http://dx.doi.org/10.5061/dryad.n4b4c > (MacGregor et al. 2017).

Results

Morphological divergence

Snout–vent length was less sexually dimorphic in non-native populations (Table 1), which was due to larger non-native females compared to native females (p < 0.001). In contrast, Head length, Greenness, Blackness, OVS blue area and Bite force were more sexually dimorphic in non-native populations (Table 1). This was also largely driven by shifts in female traits as opposed to male traits. Specifically, with the

exception of Blackness (p = 0.86), non-native females had a significant reduction in each of these traits compared to native females after accounting for SVL (p < 0.05), whereas there were no significant differences between native and non-native males (p > 0.05). Furthermore, outer ventral scale ornamentation showed no significant divergence between native and non-native males (Table 1, Supplementary material Appendix 14 Fig. A14).

Female reproductive investment

After accounting for differences in SVL, native females were heavier on average post-parturition of their first clutches than non-native females (least-squares means: native: 4.98 ± 0.15, non-native: 4.51 ± 0.12; $F_{1,16} = 5.64$, p = 0.03). Across all years and populations, non-native females produced larger and heavier first clutches than native females relative to post-parturition body mass (Fig. 1, Supplementary material Appendix 7 Table A7). Year of collection also explained significant variance in clutch mass and mean egg mass but not in clutch size (Supplementary material Appendix 7 Table A7). The probability of producing a second clutch was significantly lower for non-native females compared to native females (second clutches: 100 % of native females (39/39) and 88% of non-native females (28/32), $\chi^2 = 5.17$, p = 0.01). For females that produced both a first clutch in the wild and a second clutch in our enclosures, the duration (days) between oviposition of first and second clutches did not differ significantly between native and non-native females (native: 37.4 ± 0.7 and non-native: 38.6 ± 1.3 days, Origin: $\chi^2 = 0.58$, p = 0.45). Infertilities occurred within five first clutches (three native and two non-native) and eight second clutches (one native and seven non-native) but in only one instance (non-native) was a female's entire clutch infertile. Analyses of relative female investment in first and second clutches showed a significant interaction effect between Origin and Clutch (first or second) for all three measures of investment (Table 2, Fig. 2, also see Supplementary material Appendix 8 Table A8 for results from models excluding post-parturition body mass and including SVL). Post hoc tests revealed that the significant sources of variation were larger first clutch size (p = 0.05), and heavier first clutch mass (p = 0.03) in non-native compared to native populations, and heavier

Table 1. Summary statistics from LMMs testing for differences in the degree of sexual dimorphism between native and non-native lizards. For OVS UV Chroma and OVS Hue, where only male data are available, tests for divergence between the two origins are reported. All models included population nested within origin as a random effect and SVL as a covariate. Significant effects are highlighted in bold based on a threshold of $\alpha < 0.006$, adjusted from the nominal $\alpha < 0.05$ following Bonferroni correction for the number of tests performed on these data. SVL, Greenness, OVS blue area, Bite force, and OVS Hue were transformed (square root) prior to analysis.

	Origin	Sex	Origin × Sex	SVL
SVL (mm)	**$F_{1,24} = 13.36$, p = 0.001**	**$F_{1,1121} = 26.19$, p < 0.001**	**$F_{1,1121} = 14.66$, p < 0.001**	
Body mass (g)	$F_{1,24} = 0.20$, p = 0.65	**$F_{1,819} = 137.72$, p < 0.001**	$F_{1,819} = 4.04$, p = 0.045	**$F_{1,824} = 2268.25$, p < 0.001**
Head length (mm)	$F_{1,23} = 5.94$, p = 0.01	**$F_{1,1107} = 1875.67$, p < 0.001**	**$F_{1,1108} = 17.19$, p < 0.001**	**$F_{1,1108} = 1015.09$, p < 0.001**
Greenness	$F_{1,25} = 2.51$, p = 0.13	**$F_{1,1088} = 101.55$, p < 0.001**	**$F_{1,1089} = 19.65$, p < 0.001**	**$F_{1,1097} = 310.37$, p < 0.001**
Blackness (%)	$F_{1,14} = 0.02$, p = 0.88	**$F_{1,562} = 311.14$, p < 0.001**	$F_{1,562} = 5.88$, p = 0.02	**$F_{1,569} = 50.21$, p < 0.001**
OVS blue area (mm²)	$F_{1,12} = 8.30$, p = 0.014	**$F_{1,487} = 458.95$, p < 0.001**	**$F_{1,487} = 20.42$, p < 0.001**	**$F_{1,487} = 28.25$, p < 0.001**
Bite force (N)*	$F_{1,9} = 1.24$, p = 0.29	**$F_{1,201} = 442.59$, p < 0.001**	**$F_{1,202} = 9.09$, p = 0.003**	**$F_{1,203} = 177.41$, p < 0.001**
OVS UV chroma	$F_{1,8} = 0.21$, p = 0.66			$F_{1,89} = 0.07$, p = 0.79
OVS hue (nm)	$F_{1,8} = 0.20$, p = 0.67			$F_{1,88} = 2.20$, p = 0.14

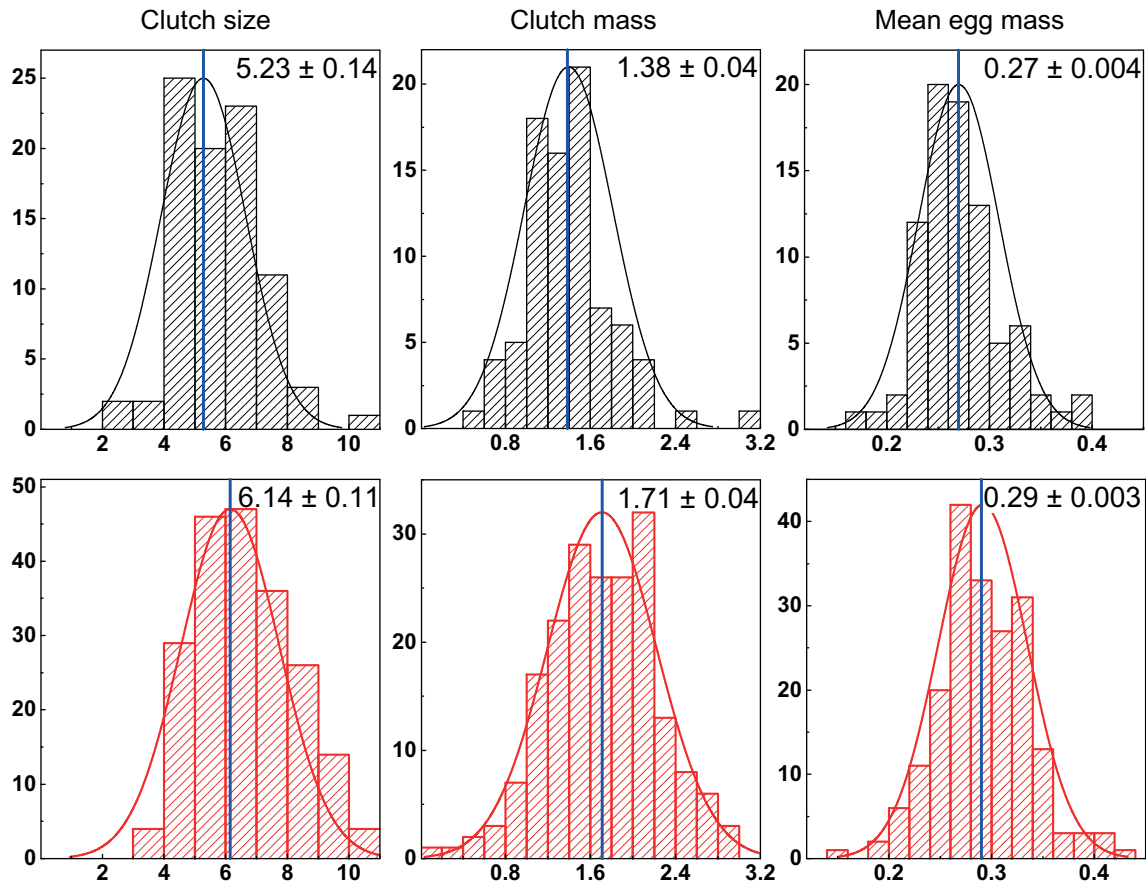*For analysis of Bite force, we controlled for body temperature at testing ($F_{1,205} = 1.43$, p = 0.23).

Figure 1. The distributions of clutch size, clutch mass (g), and mean egg mass (g) for the first seasonal clutches of native (above, black) and non-native (below, red) females collected between 2010 and 2015. For each plot, the Y-axis depicts the count of individuals and the solid blue line represents the mean value, which is reported ($\pm$ 1 SE) in the top right corner.

clutch mass in non-native first clutches compared to non-native second clutches (p = 0.005). Accordingly, mean egg mass was heavier in non-native first compared to non-native and native second clutches (p = 0.01 and 0.003, respectively).

Male reproductive investment and sexual selection

Sexual behaviour

Larger males engaged in more territorial interactions but there were no differences between native and non-native males in the numbers of competitive interactions observed (native: 14.49 $\pm$ 1.20, non-native: 13.93 $\pm$ 1.30; Origin: $\chi^2 = 0.06$, p = 0.80, Body size: $\chi^2 = 45.55$, p < 0.001, Origin $\times$ Body size: $\chi^2 = 1.30$, p = 0.25) or in the steepness of dominance hierarchies formed within each enclosure (Dominance rank: $F_{1,56} = 321.44$, p < 0.001, Origin: $F_{1,9} = 0.71$, p = 0.42, Dominance rank $\times$ Origin: $F_{1,56} = 1.33$, p = 0.25). Male

body size predicted courtships, females courted and mating success, and dominance predicted courtships and females courted; however, there were no significant differences in these relationships between native and non-native males (Table 3, 4). Overall, male–female courtship networks were significantly correlated with paternity networks within both native (Fishers combined test: $\chi^2 = 28.61$, p < 0.001, df = 8) and non-native (Fishers combined test: $\chi^2 = 29.9$, p < 0.001, df = 10) enclosures, and the range of effect sizes were similar for both origins (Supplementary material Appendix 9 Table A9 for matrix correlations by enclosure).

Opportunity and strength of sexual selection on males

There were no significant differences between native and non-native males in the estimated opportunity for sexual selection (Levene's test on mating success: $F_{1,64} = 0.47$, p = 0.49, Table 5) or the opportunity for overall selection (modified Levene's test on fertilization success: $F_{1,64} = 0.36$, p = 0.55,

Table 2. Summary statistics from tests for Origin differences in within-season female reproductive investment. Data are from females collected in 2014 and housed within experimental enclosures during their second seasonal receptive phase. Female ID nested within Population and Origin was included as a random effect in all models. Significant results are highlighted bold.

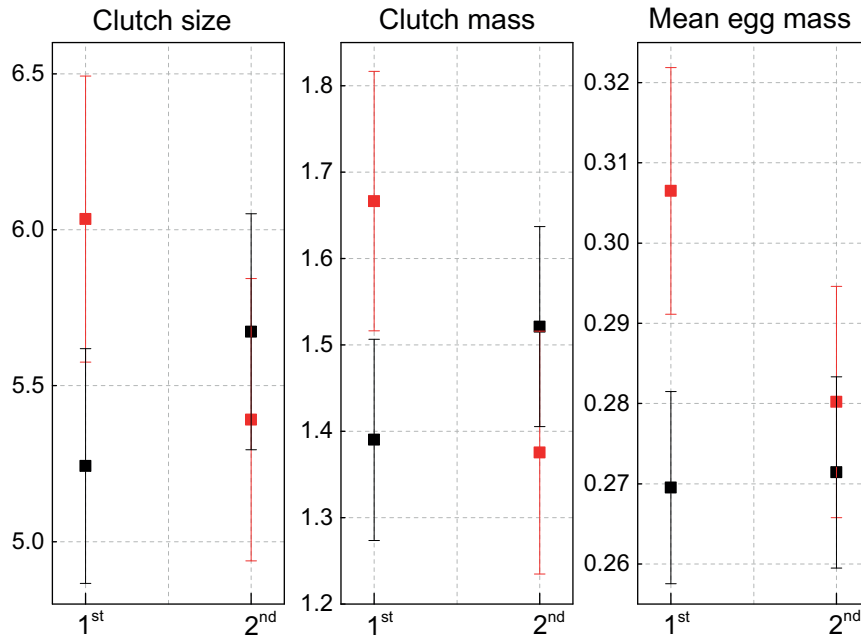|	Response	Origin	Clutch	Origin $\times$ Clutch	Post-parturition body mass
Female reproductive investment	Clutch size	$F_{1,66} = 1.35$, p = 0.24	$F_{1,64} = 0.26$, p = 0.61	$\mathbf{F_{1,66} = 6.56, p = 0.013}$	$\mathbf{F_{1,94} = 40.94, p < 0.001}$
	Clutch mass	$F_{1,69} = 0.69$, p = 0.41	$F_{1,60} = 2.21$, p = 0.14	$\mathbf{F_{1,61} = 15.05, p < 0.001}$	$\mathbf{F_{1,103} = 35.70, p < 0.001}$
	Mean egg mass	$\mathbf{F_{1,69} = 7.73, p = 0.007}$	$\mathbf{F_{1,60} = 5.33, p = 0.02}$	$\mathbf{F_{1,60} = 6.95, p = 0.011}$	$F_{1,105} = 1.65$, p = 0.20

Figure 2. Interaction plots to show the effects of female origin (native - black or non-native - red) and seasonal clutch (1st or 2nd) on three measures of female reproductive investment (clutch size, clutch mass (g), mean egg mass (g)). Data are from females collected in 2014 and housed in experimental enclosures. Effect sizes are calculated from linear mixed models including female post-parturition body mass as a main effect and female ID nested within population and origin as a random effect. Error bars depict 95% confidence intervals.

Table 5). Similarly, there was no significant difference in the Bateman gradient between native and non-native males (Table 5, Relative mating success: $F_{1,61} = 90.19$, p < 0.001, Origin: $F_{1,7} = 1.20$, p = 0.31, Origin × Relative mating success: $F_{1,61} = 0.11$, p = 0.74). Consequently, the estimated maximum intensity of selection (Jones index) was similar for males of both origins (Table 5). Furthermore, there was little evidence for a relaxation of the relationships between male sexual traits and fertilization success in non-native males (Supplementary material Appendix 10 Table A10). Levels of multiple paternity were similar within native and non-native enclosures (detected in 82% of native female clutches (Average fathers: 2.39 ± 0.15) and 70% of non-native female clutches (Average fathers: 2.44 ± 0.22): Origin: $\chi^2 = 0.00$, p > 0.99, Clutch size: $\chi^2 = 0.85$, p = 0.36). Eleven percent of native males (4/38) and 10% of non-native males (3/29) sired no offspring during the experiment.

Discussion

Direct comparisons between ancestral and descendent populations living in different climates can help to reveal both the evolutionary potential of organisms and their limits to adaptation (Kawecki 2008). Consistent with adaptive responses to the low embryo survival prospects during the later stages of the breeding season (While et al. 2015a), female wall lizards from non-native populations in England appear to shift their annual reproductive investment towards the first clutch of the season. Despite the low reproductive value of second clutches, both in terms of reduced offspring number and low offspring survival, males from non-native populations invested in territoriality and mate acquisition during their second reproductive episode to the same extent as males from the native range. Taken together, our findings suggest greater constraints on adaptive shifts in male reproductive investment compared to females in response to cooler climate.

Non-native females produce more and larger eggs from their first compared to their second seasonal reproductive episode, and when compared to the first clutch of native females. This is consistent with latitudinal patterns of investment in lizards more generally, including European lacertids (Uller and While 2014). Furthermore, wall lizards from the northern range margin in western Europe are less likely to lay second and third clutches compared to populations of the same lineage in southern France, and females from the lineage studied here (F. Aubret, T. Uller, G. While unpubl.).

Table 3. Summary statistics from tests for Origin differences in the effects of male Body size (standardized: mean = 0, SD = 1) on three measures of male reproductive investment during the second seasonal reproductive episode. Enclosure was included as a random effect in all models. Results for main effects are reported from models excluding non-significant interaction terms. Significant effects are highlighted bold.

Response	Body size	Origin	Origin × Body size
Relative number of courtships	$F_{1,56} = 9.27$, p = 0.004	$F_{1,7} = 0.07$, p = 0.80	$F_{1,56} = 0.00$, p = 0.99
Relative number of females courted	$F_{1,58} = 13.05$, p < 0.001	$F_{1,7} = 0.09$, p = 0.77	$F_{1,56} = 0.52$, p = 0.47
Relative mating success	$F_{1,58} = 15.36$, p < 0.001	$F_{1,7} = 2.17$, p = 0.19	$F_{1,56} = 0.41$, p = 0.53

Table 4. Summary statistics from tests for Origin differences in the effects of male dominance (standardized: mean = 0, SD = 1) on three measures of male reproductive investment during the second seasonal reproductive episode. Enclosure was included as a random effect in all models. Results for main effects are reported from models excluding non-significant interaction terms. Significant effects are highlighted bold.

Response	Dominance	Origin	Origin × Dominance	SVL
Relative number of courtships	$\mathbf{F_{1,56} = 5.51, p = 0.022}$	$F_{1,7} = 0.06, p = 0.81$	$F_{1,59} < 0.01, p > 0.99$	$\mathbf{F_{1,58} = 13.05, p < 0.001}$
Relative number of females courted	$\mathbf{F_{1,56} = 21.56, p < 0.001}$	$F_{1,7} = 0.11, p = 0.75$	$F_{1,59} = 0.10, p = 0.76$	$F_{1,61} = 2.30, p = 0.75$
Relative mating success	$F_{1,56} = 2.72, p = 0.10$	$F_{1,7} = 2.18, p = 0.19$	$F_{1,59} = 0.66, p = 0.42$	$\mathbf{F_{1,61} = 17.81, p < 0.001}$

This observation is indicative of female responses to seasonal time constraints on offspring survival. The greater maternal investment in first clutches observed in non-native wall lizards is plausibly an evolutionary response to strong selection for early season reproductive effort driven by direct climatic constraints on embryo development (While et al. 2015a), and the survival advantage of larger offspring (Sinervo 1990). Heritability in both clutch size and egg size has indeed been demonstrated in natural lizard populations indicating the potential for rapid evolutionary responses in reproductive investment from standing genetic variation (Sinervo and Doughty 1996, Sinervo and McAdam 2008). However, confirming the extent to which such variation in reproductive output represents genetic divergence between native and non-native populations requires the removal of environmental and maternal effects through long-term reciprocal transplant or common garden studies (Kawecki and Ebert 2004), which are logistically challenging to carry out in vertebrates.

Alternatively, but not necessarily exclusively, latitudinal shifts in reproductive investment could arise through phenotypic plasticity, initiated by environmental factors and their proximate effects on reproduction rather than adaptive genetic divergence. Climate is known to effect lizard growth and size at maturity, with cooler environments sometimes triggering larger adult body sizes at sexual maturity and, consequently, greater reproductive output (Wapstra and Swain 2001). This has been suggested to explain latitudinal variation in reproductive effort in common lizards, *Zootoca viviapara* (Roitberg et al. 2013). Nutritional state can be important for reproductive output (Olsson and Shine 1997a, Madsen and Shine 1999), thus our results may be a consequence of differences in nutritional availability between the native and non-native range and between the field verses laboratory populations. However, as differences between native and non-native females in their within-season investment were independent of female post-parturition body mass and food availability in our enclosures during the time of the experiments was very high (males gained weight during the experiment, Supplementary material Appendix 11 Table A11), reduced investment in second clutches, as observed in non-native females, is unlikely to be a passive response to resource availability. Nevertheless, raising native and non-native individuals under different climatic conditions would be necessary to rule out that ontogenetic experiences drive the population differences we observed. Even if largely due to plasticity rather than a genetic response to selection, this shift in reproductive investment may provide an important source of adaptive variation following introduction, facilitating the future genetic adaptation of females to the new reproductive environment (West-Eberhard 2003, Uller and While 2014).

In our experimental populations, non-native females investing in larger and heavier first clutches also reduced their investment in second clutches, whereas native females exposed to the same conditions during their second reproductive episode maintained, or in some cases, increased their investment. A single episode of reproduction is likely to be the optimal investment strategy for non-native females. Indeed, we found a significantly lower incidence of second clutch production in non-native females (all native females produced a second clutch). Nevertheless, still relatively few non-native females (12%) refrained from producing a second clutch. This could be explained by a rarity of genetic variation for the physiological regulation of clutch production in the native range (typically three clutches per season in Italy, the source region of the non-native animals), which, in combination with the low founder numbers for the English populations, would constrain the evolutionary potential of clutch number in non-native populations.

Territoriality, mate searching, courtship and copulation are time-consuming and energetically costly for males (Merker and Nagy 1984, Shine and Mason 2005), and carry an increased risk of predation (Cooper Jr 1999). Therefore, there should be selection against male sexual behaviour when fitness returns are negligible (i.e. as for second clutches in England; While et al. 2015a). Despite this, we found no experimental evidence that non-native males relaxed their behavioural investment in reproduction compared to males from the native range. There are several potential explanations.

Table 5. Estimates of the opportunity for sexual selection (I_s), the opportunity for selection (I), the Bateman gradient (β_{ss}) and the maximum intensity of sexual selection (S'_{max}) for native and non-native males housed within experimental enclosures during the second seasonal reproductive episode in 2014.

	n	Mating success				Fertilization success				Bateman gradient		Max intensity
		Mean (± 1 SE)	Var	I_s	$CI_{95\%}$	Mean (± 1 SE)	Var	I	$CI_{95\%}$	β_{ss}	$CI_{95\%}$	S'_{max}
Native	37	2.46 ± 0.24	2.20	0.36	0.22 0.65	5.11 ± 0.62	14.04	0.54	0.33 0.91	1.02	0.71 1.33	0.61
Non-native	29	2.28 ± 0.27	2.06	0.40	0.24 0.74	5.00 ± 0.91	24.14	0.97	0.58 1.77	0.95	0.68 1.21	0.75

Firstly, selection may in fact maintain territoriality if quality resources are an important component of male survival at all times of the year and for the re-establishment of territory occupancy between seasons. However, this does not account for our observation that non-native male dominance scores remained strongly associated with courtship behaviour during the enclosure experiment, despite a predicted relaxation in this relationship. More likely, the maintenance of sexual behaviour is triggered by cue–response systems that have been reliable in past environments (Kriska et al. 2008, Sih 2013). Since most species of lizard exhibit associated reproductive cycles whereby spermatogenesis, ovulation and mating occur synchronously (Méndez de la Cruz et al. 2015), the reproductive characters of the two sexes can be intimately linked. Consequently, courtship and mating often act as triggers for reproduction or receptivity in females (Crews et al. 1986, Manes et al. 2007), and male sexual activity can be primed by the presence of fertile females (Cooper and Perez-Mellado 2002, Head et al. 2005). If receptive females are the cues to which males respond there may be additional constraints on seasonal shifts in male behaviour if, as in wall lizards, females in non-native populations still produce second clutches (Crews and Moore 1986, also see Carretero 2006). Previous work has shown that second clutches are commonly fertile in non-native populations (Heathcote et al. 2016); hence, we did not expect to find infertility levels high enough for statistical testing. However, infertile eggs were more common in non-native females (25% non-native vs 2% native of clutches had at least one infertile egg), which may indicate a lower mating rate or reduced sperm production by non-native males (Olsson and Shine 1997b, Uller and Olsson 2005). Future work on the triggers of reproductive behaviour and its neuroendocrine basis would help to understand how the reproductive characters of the two sexes will coevolve across climatic regimes.

While environmentally driven shifts in female reproduction may relax selection on male sexual characters (Ibargüengoytía and Cussac 1999), we found no evidence that male sexual characters predict reproductive success to a greater or lesser extent in non-native populations, which corroborates our behavioural data. Furthermore, we found limited evidence for divergence in male phenotypes between native and non-native populations. The wall lizards used in this study belong to a geographically restricted lineage that show dramatic exaggeration of secondary sexual characters compared to lizards in other parts of the native range. This makes it difficult to make a meaningful comparison between males in non-native populations and populations at higher latitudes in the native range. Overall, our data may suggest that the overall intensity of selection on male traits is similar in the native and non-native populations, despite differences in the seasonality of reproductive success. Nonetheless, sexual dimorphism is generally greater in non-native populations because of reduced trait expression in non-native females. Changes in the degree of sexual dimorphism are often attributed to sexually selected exaggeration in males (Andersson 1994), but increased dichromatism due to loss of female ornamentation is supported by phylogenetic studies on birds (Burns 1998, Wiens 2001, Hofmann et al. 2008) and in dragon lizards (Ord and Stuart-Fox 2006). The causes of reduced expression in female colouration, head size and

bite force in non-native populations could involve relaxed female–female competition or, at least for colouration, increased importance of crypsis, which represent interesting avenues for future research.

In summary, we demonstrate adaptive within-season shifts in female reproductive investment in wall lizards following their recent introduction to cooler climates. Both plasticity and genetic divergence may account for these patterns. In contrast, we found no experimental evidence to suggest a corresponding loss of male investment in reproduction later in the season in non-native populations. The ability of female and male reproductive investment strategies to respond adaptively over short or long time scales will depend upon how responses in one sex affect the reliability of cues in the other. We suggest that associated reproductive cycles between males and females play a role as constraints on adaptive shifts in male behaviour.

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