

Evolving social dynamics prime thermal tolerance during a poleward range shift

Connor Wood¹, Robert N. L. Fitt^{1,2}, Lesley T. Lancaster^{1*}

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¹ School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 2TZ, United Kingdom

² Present address: Institute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZX, United Kingdom

* Corresponding author: lesleylancaster@abdn.ac.uk

Abstract

Cold tolerance plays a critical role in determining the geographical range limits of species. Previous studies have found that range shifts in response to climate warming are facilitated, paradoxically, by cold acclimation capacities, due to increasingly colder and variable weather at high latitudes. However, the evolutionary dynamics of this process are poorly understood. In this study we combined experiments and field studies to investigate the social and ecological factors affecting cold tolerances in range-shifting populations of the female-polymorphic damselfly *Ischnura elegans* in north-east Scotland, and their consequences for evolutionary change. In the field, we observed both environmental and social effects on cold tolerance and female colour morph frequencies. This process results in a latitudinal cline in female morph frequencies, due to positive feedback between social stress and thermal tolerances. Density manipulations in the laboratory provide experimental evidence that social interactions directly impact cold tolerance. Reciprocal effects of social environments on thermal acclimation may be important but commonly overlooked aspects of allee effects that contribute to the formation of range margins. Moreover, there is a wider need to consider the role of evolving social dynamics to reciprocally shape both the thermal physiology of individuals and the thermal niches of species.

Keywords

allee effects, colonization, frequency-dependent selection, group selection, indirect genetic effects, latitudinal clines, range dynamics, thermal tolerance

Significance Statement

Under periods of rapid environmental change, individuals and populations of many species may face novel thermal stressors. Here, in a range-shifting damselfly species with genetically-based social phenotypes, we show that the frequency of social phenotypes within populations can affect the ability of individuals to respond to thermal challenges. This effect is probably mediated

by a process, whereby exposure to stressful social situations activates a 'general stress response', conferring resistance to subsequent thermal stressors. Our laboratory crowding experiments support this interpretation. Our results suggest that some social systems or dynamics may be better able to facilitate the ability of animals to cope with novel thermal conditions than others, and conversely, that rapidly changing thermal conditions may impose natural selection for particular social phenotypes or group dynamics that confer thermal tolerance.

INTRODUCTION

Understanding the mechanisms shaping thermal tolerance is critical for anticipating organismal responses to anthropogenic climate change. The 20th century has seen a rise in global average surface temperatures of about 0.6 °C, with temperatures in the 21st century predicted to rise further, by between 0.6 and 4.0 °C (Field et al., 2014). Thermal tolerance plays an important role in determining a species' fundamental niche, and may therefore directly set a species' geographical range limits (Brown et al., 1996; Wiens, 2011). As the climate warms and the geographical position of their thermal range limit changes with it, species may expand their range to fill these new thermal boundaries. As a result of this process, poleward shifts in geographical ranges have already been documented in a wide range of species (Parmesan et al., 1999; Perry et al., 2005; Hickling et al., 2006; Chen et al., 2011; Mason et al., 2015).

Despite the fact that range shifts are facilitated by warmer climates, previous work suggests that species typically gain improved cold tolerance during shifts to higher latitudes and altitudes, putatively to facilitate survival in more variable, high-latitude climates (Lancaster et al., 2015; Lancaster, 2016). In fact, the capacity of a species to shift its range in response to climate change may be limited by its ability to adapt or acclimate to novel cold-weather events beyond the historical range limit (Kellermann et al., 2009; Comte et al., 2014; Diamond, 2018). Ectotherms at an expanding northern range limit are therefore subject to intense selection pressure on their cold tolerances, and may commonly exhibit evolved cold tolerances that are improved over cold tolerances observed in populations from the range core (Lancaster et al., 2015; Lancaster, 2016). Potentially by this process, terrestrial ectotherms commonly 'overflow'

their range, and occupy latitudes beyond standard predictions of their cold tolerance as measured in the centre of the species' range (Sunday et al., 2012). Understanding how range shifts both depend on and shape clinal variation in cold tolerance is therefore essential for understanding ongoing and future biodiversity shifts.

Cold tolerance clines can be created through natural selection or phenotypic plasticity, but typically by a combination of both (Schilthuizen & Kellermann, 2014). Numerous authors working on acclimation and adaptation have stressed the importance of considering phenotypic and developmental plasticity for range-shifting species (e.g. Nilsson-Ortman et al., 2012). Lande (2009) suggested that extreme changes in the environment can induce transient increases in genetic variance and phenotypic plasticity. This phase of increased adaptive potential, combined with the strong environmental effects on cold tolerance during poleward range-shifts, allows for rapid changes in phenotype and population structure. Under the beneficial acclimation hypothesis (BAH) (Wilson & Franklin, 2002; Alemu et al., 2017), and potentially due to genetic change, we should thus expect to find higher frequencies of cold-tolerant phenotypes in marginal climates at the range edge of a range-expanding species. On a physiological level, much of the adaptability in thermal tolerance can be attributed to the expression of heat shock proteins (HSPs; Dahlggaard et al., 1998; Zhao & Jones, 2012; Boykin et al., 2013; King & MacRae, 2015). Adaptive expression of HSPs is thought to be part of a generalized stress response, allowing organisms to anticipate and respond to sudden extreme stressors (Nguyen et al., 2009; Benoit et al., 2010; Shu et al., 2011) as well as to adjust to the more regular stresses endured when living under non-optimal conditions (Sørensen et al., 2003). As a result, induction of HSPs by one stressor can have a 'hardening' effect which protects organisms from subsequent stresses.

There is growing awareness that various social stresses can also cause a generalized stress response that may carry over to affect individuals' responses to a range of other, non-social stressors (Pechenik, 2006; Sih, 2011). For example, Slos & Stoks (2008) were able to induce an increase in HSP70 in *Enallagma cyathigerum* larvae through non-lethal exposure to stickleback predators. In a similar study on *Coenagrion puella* larvae, Mikolajewski et al (2007) demonstrated that predator pressure by fish, or cannibalistic pressure by conspecifics,

reduced larval growth in males and females. Moreover, in salmonid fish, dominance hierarchy formation induces HSP expression in both dominant and subordinate fish (Currie et al., 2010). However, only a few studies have investigated directly whether intraspecific social stress can induce changes in thermal tolerance. In salmonid fish, social stress experienced by subordinate fish results in poorer cold tolerance, in comparison to that in dominant fish, and despite their higher HSP expression (LeBlanc et al., 2011). In contrast, crowding stress in larval *Drosophila melanogaster* resulted in both increased HSP production and increased adult heat stress resistance (Sørensen & Loeschcke, 2001). These previous studies indicate that social stress can confer changes in thermal tolerance via a generalized stress response. However, the evolutionary dynamics of this process remain uncharacterized (but see Lancaster et al., 2017a), despite the fact that evolved differences in social phenotypes (personality) are known to correlate with population-level changes in adaptive thermal stress responses in some cases (Doering et al., 2018). If social stress can induce either beneficial or detrimental effects on thermal tolerance, and if levels of social stress can evolve, this could have major implications for thermal niche dynamics, particularly in range-expanding populations. Here we suggest that changes in social phenotypes in range-limited populations may prime cold tolerance, facilitating colonization of cooler climate areas.

Study system

The blue-tailed damselfly *Ischnura elegans* (Vander Linden, 1820) is a small, coenagrionid damselfly that is ubiquitous throughout Europe and Central Asia. They are found in a wide variety of lowland water habitats, and have exhibited recent range shifts into our study sites in the Scottish highlands (Cham et al., 2014), where the species has undertaken a range shift of 143 km northward during a recent, 60-year period; this is one of the largest recorded shifts in an Odonata species (Hickling et al., 2005). High latitudes and elevations in Scotland are associated with highly variable climates and frequent, extreme-weather events in comparison to climates in central and southern Great Britain, and *I. elegans* has not yet been able to colonize high-elevation sites within this region (Dijkstra & Lewington, 2006; Cham et al., 2014). Moreover, they remain at very low densities at higher latitudes (Hickling et al., 2005; Cham et al., 2014). Scottish populations of this

species therefore present an ideal model system to investigate the drivers of novel cold tolerance phenotypes at the range margin and near the limits of their habitat suitability. *Ischnura elegans* exhibits a genetically based, female-limited colour polymorphism (Sánchez-Guillén et al., 2005): androchromes resemble males with blue-green thorax and blue abdominal segment 8, whereas infuscans (olive-green thorax, brown segment 8) and infuscans-obsoleta (brown thorax and segment 8) are visually distinct from males and are collectively referred to as gynochromes (Cordero et al., 1998). Female morphs exhibit divergent behaviours and life histories (Van Gossum et al., 2001; Sánchez-Guillén et al., 2017), and site-specific colour morph frequencies have been shown to exhibit negative frequency-dependent dynamics (Takahashi et al., 2014; Le Rouzic et al., 2015) because common morphs exhibit decreased fecundity due to mating harassment under their scramble competition mating system (Van Gossum et al., 2001; Gosden & Svensson, 2007). Moreover, androchromes may receive additional protection from harassment due to their resemblance to males (intersexual mimicry; Gosden & Svensson, 2009). Male–male competition for food and mates can, in turn, be a powerful selective agent on male damselflies (Gosden & Svensson, 2008). Thus, social stress is a powerful evolutionary force which can cause rapid changes in both population size and allele frequencies in this species (Svensson et al., 2005; Gosden & Svensson, 2009).

We previously identified a latitudinal cline in cold tolerance in Swedish populations of *I. elegans* (Lancaster et al., 2015, 2016, 2017a; Dudaniec et al., 2018). Individuals from range-edge populations showed significantly faster chill coma recovery than core individuals. Chill coma recovery time was correlated with the minimum temperature experienced within the last 7 days, but only in range-limit populations, and not in the range core, suggesting that thermal acclimation abilities had evolved during the course of the expansion (Lancaster et al., 2015). Moreover, we also previously found that social feedback may play a critical role in the clinal variation of both cold tolerance and female colour morph frequencies for *I. elegans*. For androchromes, among-population variation in cold tolerance was best explained by recent cold weather events – a plastic response to thermal conditions consistent with the BAH. For gynochromes, however, cold tolerance was positively correlated with gynochrome frequency in

the population, suggesting a social feedback mechanism by which social stress regulates cold tolerance in these female morphs (Lancaster et al., 2017a). Moreover, the continent-wide latitudinal cline in morph frequencies for this species (increasing androchromes with latitude; Gosden et al., 2011) was found to be reversed at the range limit, where a high frequency of gynochromes was found at the most marginal or recently colonized sites (Lancaster et al., 2017a). This suggests a beneficial feedback between gynochrome frequency, social stress and cold tolerance that, under colder conditions, outweigh the typical frequency-dependent disadvantage of high gynochrome frequencies accruing from male-mating harassment. We previously suggested that the social stress involved with male-harassment may have had a cold tolerance priming effect in *I. elegans*, and as gynochromes lack the protection from male-mimicry and are thus particularly susceptible to higher harassment rates, they become subject to positive frequency-dependent selection based on their more readily stress-induced phenotype at the colder range limit (Lancaster et al., 2017a).

In this study we investigated the alternative social and environmental mechanisms by which thermal tolerance is inducible in Scottish range-shifting populations of *I. elegans*, to test for parallel and novel social–thermal evolutionary responses across a new geographical expansion gradient. We also conducted experimental manipulations of the social environment in the laboratory to infer causation. Thermal tolerances were assessed at both larval and adult stages for wild-caught individuals from a geographically dispersed range of study sites, each within 100 km of the species’ elevational range margin, in order to examine the influence of environmental and social factors on cold tolerance across a range of social and environmental conditions. Furthermore, social stress was generated via experimental social crowding of larvae in the laboratory, and thermal tolerances were then assessed to identify whether social crowding induced changes in cold tolerance in comparison with isolated individuals. We hypothesized that both colder climates and social crowding would increase the cold tolerances of damselflies during larval and adult stages. Based on our previous findings, we further expected that in females, gynochrome morphs would show a stronger relationship between social factors and cold

tolerance than androchromes, and that this process may drive clinal variation in morph frequencies at the range limit.

MATERIAL AND METHODS

Social crowding study

A total of 161 individual *I. elegans* larvae of a variety of developmental stages were captured for the social crowding study, between 5 and 18 May 2017, prior to the 2017 breeding season (i.e. ensuring all larvae were at least 1 year old). Larvae were sampled from Midmar Stillwater Fishery (for site information, see Table 1; Fig. 1). Larvae were collected from this single site in order to reduce any confounding variability in larval thermal tolerances due to developmental conditions, which may differ across heterogeneous sites. Larvae were captured via dip netting and an initial identification was made based on Cham (2012). *Ischnura elegans* larvae were transferred to plastic containers with air holes for transport back to the laboratory at the University of Aberdeen. Further identification and measurement were conducted using a Yenway SZN71 stereo microscope with a YenCam10 microscope camera (Ningbo Sunny Instruments Co. Ltd, Zhejiang, China), and images were analysed with YenCam software (v.3.6.9). Head width (widest distance between the outer margins of the eyes; mm) and number of caudal lamellae were recorded for each individual. All damselfly larvae have three caudal lamellae at birth, but some may be lost during development or capture. Caudal lamellae function as gills, so loss of lamellae may lower a damselfly larva's tolerance to heat and oxygen stress (Verberk & Calosi, 2012). Following the methodology of previous damselfly larvae research (e.g. Rolff, 1999), head widths were used as an indicator of larvae size, as head widths are well correlated with overall body size.

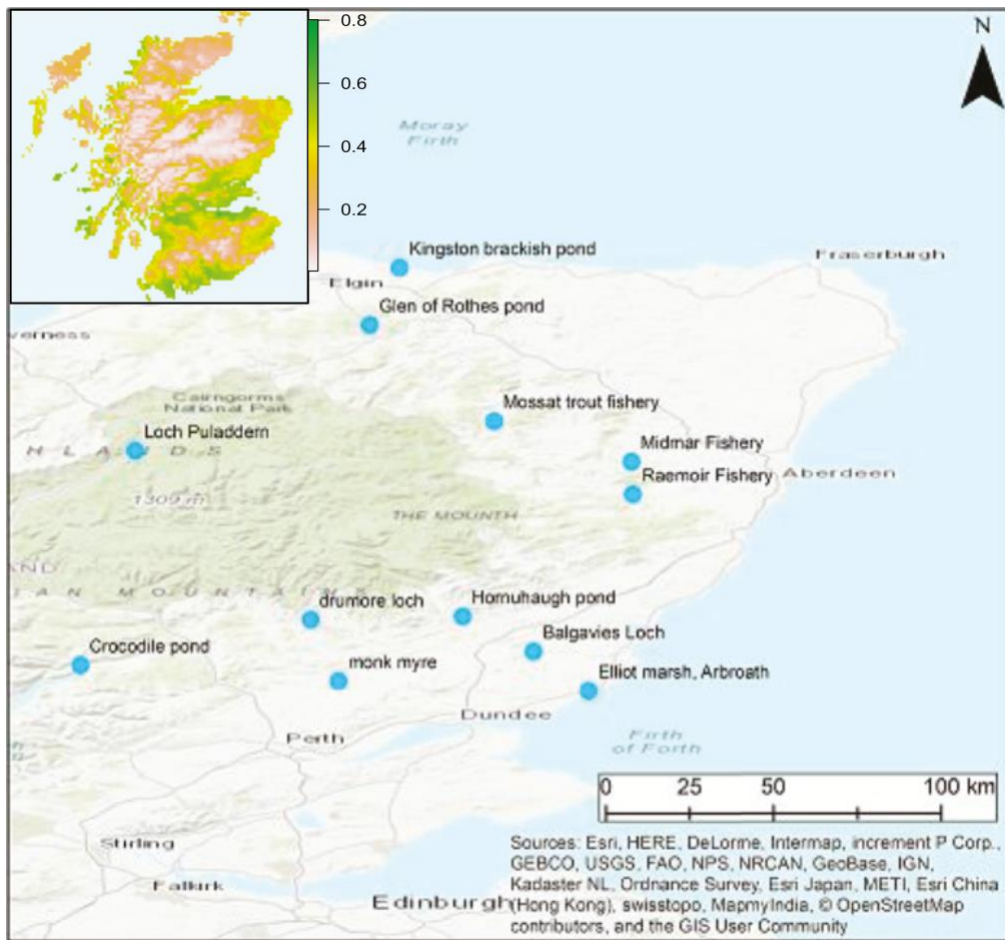
Table 1.

Location and *Ischnura elegans* habitat suitability values (from Fitt & Lancaster, 2017) for our 12 study sites near the northern range limit in Scotland. Density estimates are individuals captured per minute.

Site	Latitude (°N)	Longitude (°W)	Elevation (m)	<i>I. elegans</i> habitat suitability	<i>I. elegans</i> density in 2017
Drumore Loch	56.731	3.366	403	0.09	0.54
Midmar trout fishery	57.156	2.507	188	0.23	0.34
Mossat trout fishery	57.265	2.875	203	0.25	0.25
Raemoir fishery	57.069	2.504	85	0.44	0.61
Hornuhaugh pond	56.742	2.959	249	0.44	0.13
Loch Puladdern	57.186	3.837	253	0.45	0.74
Glen of Rothes pond	57.522	3.208	66	0.45	0.05
Balgavies Loch	56.648	2.770	84	0.51	0.16
Elliot Marsh	56.542	2.622	9	0.57	0.14
Crocodile pond	56.612	3.984	119	0.58	0.10

Monk Myre	56.569	3.291	50	0.60	0.53
Kingston brackish pond	57.676	3.127	3	0.63	0.19

Figure 1.



Locations of study sites near the elevational range limit for *Ischnura elegans* in Scotland. Inset shows habitat suitability for this species, with more suitable areas depicted in green (details of *I. elegans* habitat suitability estimation reported in Fitt & Lancaster, 2017). Map created using Arcmap (ESRI 2012; Redlands, CA, USA).

After collection and measurement, *I. elegans* larvae were randomly assigned to one of two social crowding treatments and were transferred to cylindrical enclosures (diameter 6.75 cm, height 3.5 cm) filled with 45–50 mL of aged (24+ h) tap water. The enclosures were sealed, although air holes in the lids permitted oxygen transfer. A fine mesh wall divided the enclosure into two compartments. Each compartment was roughly identical in size and contained a small section of plastic aquarium plant. Larvae in the ‘Isolated’ treatment were kept to a single compartment, while the other remained empty. In the ‘Paired’ treatment, two size-matched larvae were transferred to the same enclosure, with one in each compartment. By separating individuals in the same waterbody with the mesh wall, the individual larvae were thus exposed to the visual and chemical stimuli of their conspecifics in a contained environment whilst also being prevented from performing direct agonistic interactions such as cannibalism. Additionally, rearing the Isolated larvae in identical mesh enclosures ensured all larvae were exposed to the same physical environmental conditions during the study. Eighty-one larvae were randomly assigned to the Isolated treatment and 80 to the Paired treatment.

Feeding occurred approximately every 24 h, with larvae being fed two or three live aquatic invertebrates [e.g. brine shrimp (*Artemia*) bloodworms (*Glycera*), and *Gammarus*; these food items were offered in rotation to all individuals every day to every few days as available from local suppliers and our own rearing apparatus – all individuals were subject to the same feeding regime]. Water in each replicate was aerated during feeding, and approximately 20% water changes occurred every 5–7 days, or more frequently if required. Enclosures were maintained at 15 °C in a refrigerated incubator for the duration of the study, which approximates summer freshwater temperature in Scotland (Hrachowitz et al., 2010). The larvae were reared in these enclosures until 19 June (32–45 days, depending upon capture date). At the end of the rearing treatment, 39 individuals from the Isolated treatment and 40 individuals from the Paired treatment survived for cold tolerance trials.

Social and environmental determinants of cold tolerance in the wild

We sampled a total of 856 damselflies during June 2017, conducting repeat visits to 12 pond/loch sites throughout north-east Scotland near the northern range limit for this species (Fig. 1; Table 1). The sites chosen were characterized as permanent water bodies with open, shallow bottoms and consistent varieties of emergent vegetation. They covered a large spatial extent and a range of climatic variation: latitude ranges from 56.37° to 57.68°N and longitude from 4.13° to 2.11°W, with elevation ranges from 3 to 442 m. The selected sites had previously been studied for their established *I. elegans* populations, with population density estimates available for the years 2014–2016 (data deposited in BioTIME: Dornelas et al., 2018). In total, we sampled 551 *I. elegans* which we used to calculate sex ratio, population density and female morph frequencies at each site. Across all sites, the 551 *I. elegans* comprised 247 males (44.82%), 196 females (35.57%) and 108 larvae (19.6%). Larvae were all at least 1 year old (resulting from the previous reproductive season or earlier). Of these 551 individuals, we assessed cold tolerance for N = 375 *I. elegans*, including 160 adult females [N = 82 androchromes, N = 36 gynochromes (86% *infuscans-obsolata*), N = 42 *violacea*], 107 adult males and 108 larvae.

Cold tolerance trials

Thermal tolerance for adult and larvae damselflies was measured as the critical thermal minimum (CT_{min}; i.e. the temperature at which individuals lost muscle control). CT_{min} was assessed using a thermal ramping protocol. Damselflies were placed individually in test tubes which were suspended in a Grant TX150 programmable circulating water bath with C2G cooling attachment (Grant Instruments, Shepreth, UK). Adults were placed in 50-mL centrifuge tubes open to the air, and larvae in tubes filled with dechlorinated tap water at 15 °C. After a 10-min equilibrium period at 15 °C, the water bath temperature was gradually decreased at a rate of 0.1 °C min⁻¹ until loss of movement and responsiveness was observed (individual no longer responded to prodding). Once CT_{min} had been recorded, damselflies were removed from the water bath, and individuals from both the wild and the rearing experiment were measured. Larval head widths were recorded as described above. Adult body length was assessed by scanning individuals on an Epson perfection photo scanner (Suwa, Nagano, Japan), and analysing images with Fiji software (Schindelin et al., 2012).

Data analysis

We analysed the relationships between CT_{min} and social/environmental factors for freshly captured individuals across a range of sites, to test for the potential role of social interactions on thermal tolerance for *I. elegans* in the wild. Separate linear mixed models were run for adult and larval wild-caught damselflies, where each model included effects for individual traits, as well as social and environmental predictors. Social predictors included population density, sex ratio and colour morph frequencies. These were estimated for the *I. elegans* population at each site using the capture data in the current year (2017). Density estimates were calculated as the number of individuals captured per minute of catching effort (CPUE; Svensson et al., 2005; Lancaster et al., 2015). Sex ratio and morph frequency estimates were calculated under the assumption that catchability did not differ significantly between males, females and colour morphs (Stoks, 2001), and thus CPUE was representative of the relative abundances of each sex/morph. Sex ratio was calculated as the proportion of males in the adult population per site. Colour-morph frequencies were estimated as the number of androchromes divided by the number of androchromes + gynochromes caught per site. Data on *I. elegans* site density estimates from 2016 were also included in models to test for effects of population densities during development on thermal tolerances (in the study region, *I. elegans* can take as long as 3 years to mature, with most maturing in 2 years; Fitt et al., 2018).

These social effects were contrasted with potential climatic effects on CT_{min}. To model climatic effects, we included site-specific values for habitat suitability, which was estimated using Maxent models for a previous study (Fitt & Lancaster, 2017), and including nine non-correlated bioclimatic variables (Hijmans et al., 2005), of which bio1 (mean annual temperature) had the highest permutation importance (54%) followed by bio10 (mean temperature of the warmest quarter; 15.5%) and bio2 (mean diurnal temperature range; 11%). To identify recent acclimation effects on thermal tolerance, we also included an estimate of the minimum temperatures recorded in the region over the 6 days prior to capture (data from accuweather.com). In addition to social and environmental factors, we also included fixed effects for the individual traits body size (head width for larvae, body length for adults) and sex (adult analysis only).

We included all fixed terms and their interactions and performed corrected Akaike information criterion (AICc) comparisons of different hypothesized combinations of social and environmental determinants of cold tolerance, to arrive at best-fit models. Analyses were performed in the lme4 and lmerTest packages for R v.3.5.0 (R Core Development Team, 2012; Bates et al., 2014; Kuznetsova et al., 2014). AICc was calculated using the AICcmodavg package for R (Mazerolle, 2015). Collinearity of fixed effects was assessed using the vif.lmer() function for R (Frank, 2014). Site ID and capture date were included as random factors in all models. CTmin was log-transformed [$\log(\text{CTmin} + 2)$] to improve normality and homogeneity of the residuals for the models. For adult females, we also conducted a targeted hypothesis test to identify if any of our social or environmental predictors affected cold tolerance differentially among female morphs. For this, mixed models were run as described above, but the analysis was limited to adult females only. We examined each social and environmental factor for significant interactions with female colour morphotypes. Non-significant interactions (and their associated main effects) were sequentially removed from the analysis. Using data on morph frequencies, population densities and sex ratios gathered in 2014 from 93 sites across the study region (Fitt & Lancaster, 2017), we also examined relationships between social and environmental predictor variables using linear mixed models in R, and including latitude and longitude to investigate spatial trends.

For the social crowding study, we analysed the drivers of CTmin using linear mixed models as described above, in which treatment (Paired vs. Individual), head width, numbers of caudal lamellae (1–3) and all interactions were included as fixed effects. A random effect for container was also included in the models. To assess the potential effects of hypoxia on thermal tolerance, we analysed existing data from 20 damselflies from a previous study which used the same rearing protocol described here, but which explicitly manipulated oxygenation levels (G. Morrison & L. T. Lancaster, unpubl. res.). All data associated with this study are included in the online supporting information ([Supporting Information, File S1](#)).

RESULTS

Across sites, CTmin of adult male, adult female and larval *I. elegans* captured from the field were 3.92 ± 0.32 , 3.15 ± 0.26 and 2.13 ± 0.22 °C (mean \pm SE), respectively. The best model explaining cold tolerance of wild-caught, adult damselflies from our 12 sites (Table 2A) included a significant three-way interaction among sex, habitat suitability and sex ratio, and a two-way interaction between sex and site density in the current year. Male cold tolerance was best explained by the interaction between habitat suitability and population sex ratio, such that males from poor-quality sites had lowest (best) cold tolerance overall, but cold tolerance was also lower (better) at high-quality sites when the proportion of males in the population was high (Fig. 2A; effect of habitat suitability \times sex ratio in males only = -18.82 ± 4.72 , $t = -4.21$, $P = 0.006$, this effect was not significant in females). In contrast, female cold tolerance was best explained by population density, such that female cold tolerance improved (was lower) with higher population density (Fig. 2D; effect of density in females = -0.69 ± 0.22 , $t = -3.19$, $P = 0.002$, this effect was not significant in males) and with lower habitat suitability (Fig. 2B; effect of habitat suitability in females = 1.88 ± 0.41 , $t = 4.64$, $P = 0.0005$). No other trait or social or environmental effect significantly affected cold tolerance.

Table 2.

Fixed effects in best-fit models for adult (A) and larval (B) *Ischnura elegans* cold tolerances, in wild-caught individuals from our 12 range-limit populations

	Effect	SE	t	P	VIF
A: Best model for adult <i>Ischnura elegans</i> cold tolerance:					
Androchrome frequency	-0.91	0.45	-1.7	0.11	1.35
Site density current year (<i>I. elegans</i>)	-0.67	0.22	-3.01	0.003	1.12

Sex (M)	2.34	1.09	2.15	0.03	1.05
Sex ratio (% M)	-2.31	2.37	-0.98	0.34	1.75
Habitat suitability	4.7	2.65	1.77	0.09	1.45
Density × Sex	0.67	0.3	2.26	0.03	
Habitat suitability × Sex	-6.72	2.56	-2.64	0.009	
Habitat suitability × Sex ratio	6.24	5.34	1.17	0.25	
Habitat suitability × Sex ratio × Sex	-13.27	5.26	-2.52	0.01	

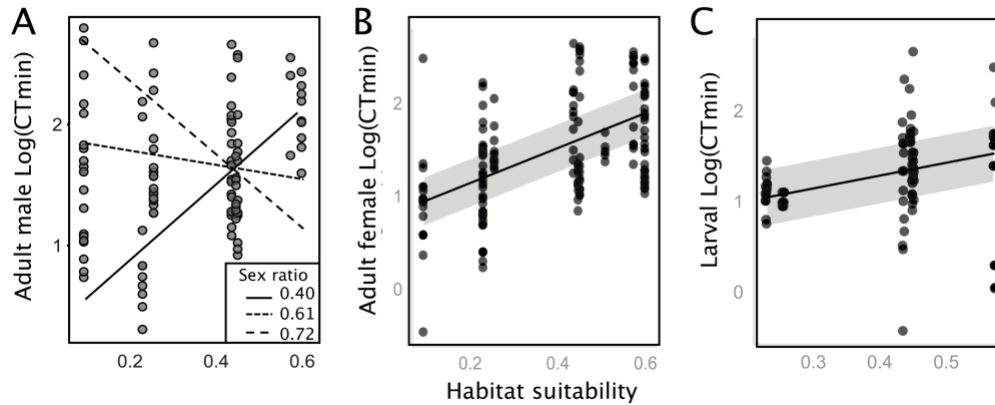
B: Best model for larval *Ischnura elegans* cold tolerance:

Site density previous year (<i>I. elegans</i>)	-0.06	0.04	-1.87	0.067	1.14
Habitat suitability	1.42	0.72	1.96	0.065	1.14

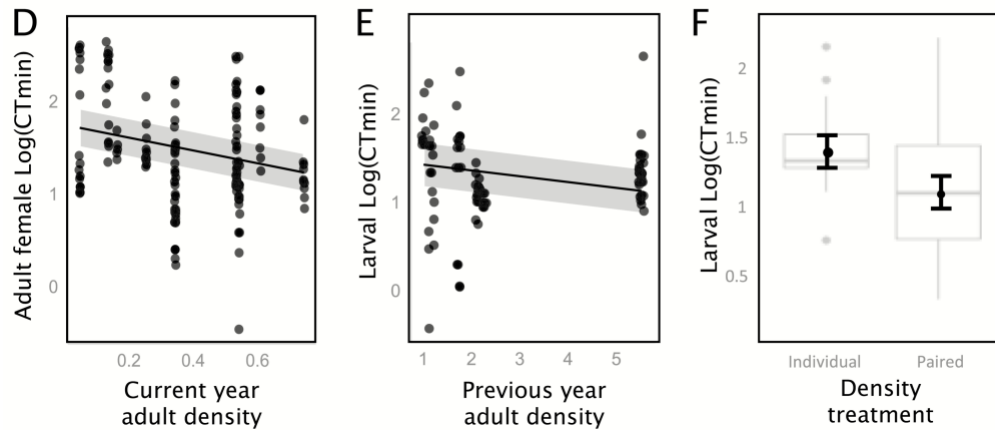
VIF, variance inflation factor.

Figure 2.

Environmental effects on cold tolerance



Social effects on cold tolerance

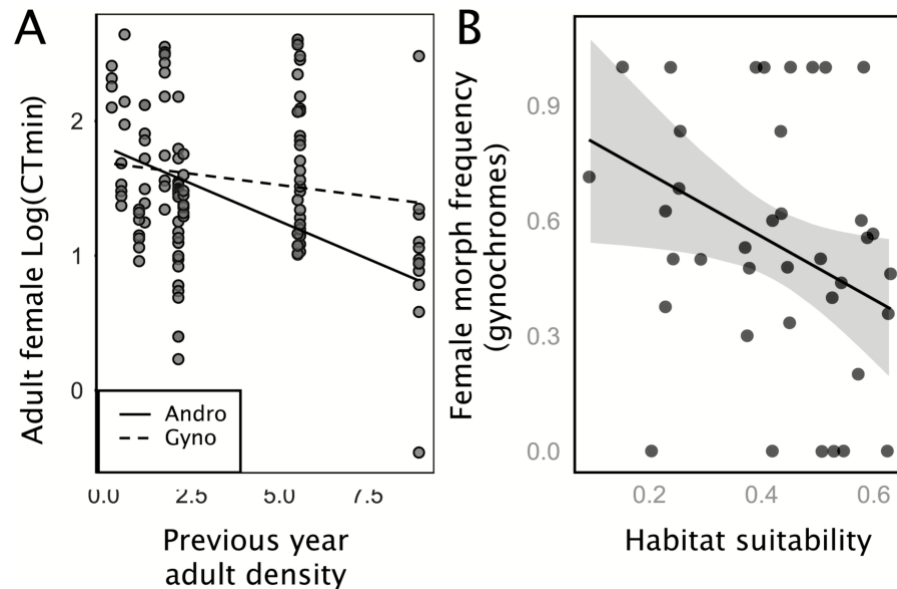


Environmental (A–C) and social (D–F) determinants of cold tolerance in adult and larval *Ischnura elegans* captured near their elevational range limit in Scotland.

Corroborating previous findings in Swedish populations of *I. elegans*, we found that gynochrome females exhibited stronger social controls on cold tolerance than androchromes, such that gynochrome females exhibited improved cold tolerance after having developed in high-density sites, whereas androchromes were unaffected by developmental densities (effect of previous year population density \times female morph = -0.11 ± 0.04 , $t = -2.99$, $P = 0.003$; Fig. 3A). No other social or environmental factor interacted with female colour morphotype to affect cold

tolerance, and, as in Swedish populations, there were no clear, absolute differences in the cold tolerances of these alternative colour morphs, suggesting that the alternative morphs differ in drivers of acclimation processes but not in their absolute capacities to express cold tolerance.

Figure 3.



A, differential effects of developmental densities on adult thermal tolerances by morphotype; gynochromes are more likely to experience beneficial social priming. B, gynochromes are more frequent in lower-suitability sites near the elevational range margin, suggesting that social priming effects on gynochrome cold tolerance are adaptive there, and contribute to evolved morph frequency shifts.

Cold tolerances of wild-caught larvae were best explained by a model which included habitat suitability and population density in the previous year (Table 2B). As in adults, larval cold tolerance was lower (better) in sites characterized by lower habitat suitability (Fig. 2C). Wild-caught larval thermal tolerance was also improved (lower) in sites characterized by high developmental densities (previous year adult densities; Fig. 2E), corroborating our experimental

findings (see below) that larval competition has a beneficial effect on cold tolerance. No other social or environmental factor influenced larval cold tolerances. Body size did not significantly affect cold tolerance in either adults or larvae.

Similar to evolved social processes detected at the range margin in Sweden (Lancaster et al., 2017a), we also found that gynochrome frequencies were higher in cooler, less suitable habitats among range-limit sites in Scotland (effect of habitat suitability on gynochrome frequency = -0.81 ± 0.39 , $t = -2.10$, $P = 0.04$; Fig. 3B), thus confirming that parallel shifts in the frequencies of social–thermal phenotypes, putatively mediated by social stress, have evolved over two independent range expansion trajectories. Also consistent with Swedish populations of *I. elegans* (Lancaster et al., 2015), we find that Scottish populations of *I. elegans* tend to show higher population densities at more suitable sites [effect of Maxent-derived habitat suitability on $\log(\text{density}) = 0.27 \pm 0.12$, $t = 2.33$, $P = 0.02$].

The best model for cold tolerance of larvae from our rearing experiment included only a single fixed effect for social crowding treatment (effect of pairing vs. isolation = -0.27 ± 0.08 , $t = -3.40$, $P = 0.001$). Individuals reared in the paired treatment experienced significantly improved (lower) cold tolerance in comparison to individually housed larvae (Fig. 2F; individual larvae expressed a CT_{min} of 2.23 ± 0.19 °C, while paired larvae expressed a CT_{min} of 1.40 ± 0.24 °C). The effects of body size and number of tail segments were not significant and worsened model fit. There was no effect of dissolved oxygen levels within containers on the thermal tolerance of larvae (effect of dissolved O₂ concentration on cold tolerance = -0.92 ± 1.43 , $t = -0.64$, $P = 0.53$). Additionally, for individuals in the Paired treatment, relative and absolute differences in head width between paired conspecifics had no significant effect on CT_{min}. There was also no significant difference between treatments in head width, number of caudal lamellae, growth rate or treatment duration ($P = 0.489$, 0.78 , 0.328 and 0.07 for head width, lamellae, growth rate and treatment duration, respectively).

DISCUSSION

We investigated social and environmental drivers of cold tolerance in marginal populations of a range-shifting damselfly, addressing the hypothesis that evolved social factors can influence colonization of stressful environments. We posited that these evolved social interactions can initiate a generalized stress response that offers protection against novel climatic variation, in addition to the standard cold acclimation processes that can evolve in response to cooler or more variable climates, i.e. where prior exposure to cold weather primes individuals to express adaptive cold tolerances (Lancaster et al., 2015). As expected, and in accordance with the BAH, all individuals exhibited better tolerance to cold when they were captured from sites characterized by lower climatic habitat suitability (i.e. generally cooler and more climatically variable sites; see Methods and Fitt & Lancaster, 2017; Fig. 2A–C), in addition to experiencing social effects on cold tolerance (see below). The effect of habitat suitability on cold tolerance is likely to reflect the species' capacity for phenotypic plasticity in the form of thermal acclimation (Lancaster et al., 2017b), but may also contain some element of local adaptation in cold tolerances. A recent population genomic study of these populations revealed generally weak genetic structure and low genetic divergence across the region, and also suggested high levels of gene flow among most sites (R. N. L. Fitt and L. T. Lancaster, unpubl. data). Specifically, significant genetic differentiation was observed at a few isolated, high-elevation sites (notably Loch Puladdern and Honuhaugh Pond; Fig. 1), sites which were also associated with atypical values for climatic variability. Moreover, in a separate region, we found significant genetic differentiation in genetic variants mapping to HSP70 across the climatic gradient over which *I. elegans* has recently expanded (Dudaniec et al., 2018), as well as latitudinal variation in cold tolerance plasticity (Lancaster et al., 2015). More work is needed to disentangle the genetic and plastic components of the effect of habitat suitability on cold tolerance of these range-shifting damselflies.

Interestingly, we also found evidence for significant social influences of the expression of cold tolerance in larvae and adults of both sexes within range-limit populations of this species (Fig. 2D–F). For adult male *I. elegans*, cold tolerance was best explained by an interaction between sex ratio and habitat suitability, such that males typically exhibited better tolerance to cold following

direct acclimation to cooler climates (see above), but in the absence of direct acclimation to cold weather, they could also achieve improved cold tolerance when at high frequency in the population. Males are likely to experience social stress at high frequency due to increasingly intense competition for mates (Cordero et al., 1997), or due to other forms of male–male interference for feeding or basking sites (Baird & May, 2003; Fitt & Lancaster, 2017), and this source of social stress evidently is associated also with increased resistance to cold. In contrast to males, both females and larvae experienced increased cold tolerance in response to high population density. Social stress associated with high densities probably reflects both increased predation pressure and competition for food, basking and oviposition sites (Pechenik, 2006). For adult females, high densities also reflect more intense mating harassment overall (Golubović et al., 2018). Van Gossum et al. (2001) found that mating harassment rates, and particularly the tendency of males to target common female morphotypes, increased at high population densities, suggesting that common morphs, and particularly gynochromes (Rivera & Sánchez-Guillén, 2007), will be most prone to experiencing the cold tolerance benefit of density-induced social stress.

Moreover, the current study demonstrates experimentally that crowding has a direct, beneficial effect on cold tolerance expression in larval damselflies. As our study design prohibited the role of agonistic interactions by separating the individuals into compartments, we can infer that the effect on cold tolerance was caused by indirect stress due to visual or chemical cues. Previous studies have demonstrated that damselfly physiological stress levels and HSP production are mediated by visual cues of conspecifics and predators (McPeck et al., 2001; Slos & Stoks, 2008). Analysis of the oxygenation dataset confirmed that hypoxia has no significant effect on cold tolerance *for I. elegans* larvae. These findings are consistent with previous research (Boardman et al., 2016). While some studies have demonstrated that anoxia can induce cold-hardening (in flies; Coulson & Bale, 1991; Nilson et al., 2006), even if this were the case for *I. elegans*, the water in each enclosure was aerated frequently enough to discount anoxia as an influential factor. The increased cold tolerance for the paired larvae can therefore be best explained as a physiological response to chronic social stress.

As in Sweden (Lancaster et al., 2017a), we found that the continental-wide cline of increasing androchrome frequency with latitude was reversed at the northern range limit in Scotland (Fig. 3B). Scottish range limit sites characterized by the lowest habitat suitabilities were dominated by the gynochrome morphs (*infuscans* and *infuscans-obsoleta*). Moreover, as in Swedish range-limit females, we find that Scottish range-limit gynochromes are more susceptible than androchromes to beneficial effects of social stress on the expression of their cold tolerances. In the current study we find that gynochrome females that are at high density during development were more likely to exhibit improved cold tolerance as adults, whereas androchromes were less likely to receive this social benefit (Fig. 3A). If social stress is an important source of phenotypic variation in cold tolerance, the differences in social sensitivity among these morphs may explain why gynochromes reach high frequency at the range margin. Moreover, increased gynochrome frequency at the population level can exacerbate the stress levels experienced by individual gynochromes, via increased male harassment rates (Van Gossum et al., 2001; Lancaster et al., 2017a). This suggests that at the range limit, the typical negative frequency-dependent selection on female morph frequencies is transformed into positive frequency-dependent selection, because social stress shifts from having a negative effect on fitness (in the range core), to conferring an adaptive cold tolerance benefit at the range edge, where social stress accrues a fitness benefit. By this mechanism, evolved shifts in colour morph frequency during range shifts can potentially promote the colonization of cooler habitats at the range margin, extending the geographical extent of the range shift.

Although populations were likely to reach higher density at greater levels of habitat suitability (i.e. further from the range margin), all of our sites are within 100 km of the range margin in Scotland, and high gene flow across the region suggests that this species is not dispersal-limited across any of our study sites (R. N. L. Fitt and L. T. Lancaster, unpubl. data). Therefore, populations persisting at the lowest levels of habitat suitability are likely to represent sink habitats which are consistently replenished by individuals from more suitable sites. The results presented here suggest that the survival of immigrants is likely to be positively density-dependent, where relatively larger colonizing population sizes confer a survival advantage to cold weather events;

gynochromes are more likely to reap this social benefit and thus to achieve higher frequencies in these habitats.

The observed requirement for social dynamics to augment cold tolerance at the range margin supports the idea that a species' thermal acclimation potential is not always sufficient alone to cope with novel climatic variation (Gunderson & Stillman, 2015; Lancaster et al., 2017a). Moreover, the thermal acclimation benefits of social interactions reported here may be an important but commonly overlooked aspect of allee effects which contribute to the formation of range margins (Sexton et al., 2009). These results also highlight the need for further research into the extent to which social or sexual systems and group dynamics contribute to the thermal physiology of individuals, and the thermal niches of species.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

File S1. Supporting data for this study.

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Author contributions: CW conceived the idea for the study in discussion with LL. CW and LL developed the experimental design and conducted the fieldwork. CW conducted the

experiments. CW and LL analysed the data. CW drafted the manuscript. RF contributed to previous data collection and generating the habitat suitability layer. All three authors contributed critically to the drafts and gave final approval for publication.

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