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Do group dynamics affect colour morph clines during a range shift?

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Abstract

Species exhibiting colour-polymorphism are thought to have an ecological advantage at the landscape scale, because spatial segregation of alternatively-adapted ecotypes into diverse habitats can increase the total species' niche breadth and thus confer greater geographic range size. However, morph frequencies are also influenced by intra-populational processes such as frequency- or density-dependent social interactions. To identify how social feedback may affect clinal variation in morph frequencies, we investigated reciprocal interactions between morph-specific thermal tolerance, local climatic conditions, and social environments, in the context of a colour-morph frequency cline associated with a recent range expansion in blue-tailed damselflies (*Ischnura elegans*) in Sweden. Cold tolerances of gynochromes (female-like female morph) were positively correlated with local gynochrome frequencies, suggesting a positive frequency-dependent fitness benefit of being common. In contrast, androchrome (male-mimic female morph) cold tolerances were improved following recent exposure to cold weather, suggesting a beneficial environmental acclimation effect. Thus according to an environment-matching hypothesis for clinal variation, androchrome frequencies should therefore increase towards the (cooler) range limit. In contrast to this prediction, gynochrome frequencies increased at the expanding range limit, consistent with a positive frequency-dependent social feedback when invading novel climates. Our results suggest that when phenotypes or fitnesses are affected by interactions with conspecifics, beneficial social effects on environmental tolerances may i) facilitate range shifts and ii) reverse or counteract typical patterns of intraspecific interactions and environment-matching clines observed in stable populations observed over broader geographic scales.

Keywords: thermal tolerance plasticity, group selection, frequency-dependent selection, indirect genetic effects, social feedback, climate change, insects, expansion front, clinal variation, local adaptation

Introduction

Many species exhibit conspicuous colour polymorphisms, in which individuals within an interbreeding population exhibit alternative colours, which are often associated with alternative social, reproductive and ecological strategies (Sinervo & Lively, 1996; McKinnon & Pierotti, 2010; Svensson, 2017). These alternative types, segregating within populations, are often highly differentiated from each other along multiple trait axes, and thus represent alternatively co-adapted trait complexes within an interbreeding population (Sinervo & Svensson, 2002; Lancaster *et al.*, 2007; Svensson, 2017). Stable polymorphisms might be adaptive in reducing intraspecific competition, because alternative morphotypes (morphs) use differing tactics to access mates or avoid enemies, and alternative morphs may also specialize on different resources in the environment (Skulason & Smith, 1995; Lancaster *et al.*, 2010; Berggren *et al.*, 2012). Colour polymorphisms might result in broader niches at the population level, and could resolve generalist/specialist trade-offs, if a broad population niche is partitioned among multiple, more specialized individual strategies (Bolnick *et al.*, 2003).

Polymorphisms may also be beneficial to species at regional scales, if sorting of different morph frequencies among populations along a geographic cline allows the species to occupy a broader range of habitats across the landscape, resulting in a larger species range sizes (Galeotti & Rubolini, 2004; Forsman & Aberg, 2008; Berggren *et al.*, 2012). Spatial variation in population morph frequencies can also facilitate range shifts under changing environments, if some morphs are preadapted to novel habitats as they open up for colonization (West-Eberhard, 1986; Shine *et al.*, 2011; Berthouly-Salazar *et al.*, 2012). However, because expression of morph-specific traits and morph fitnesses commonly depend on local demographic factors (i.e., alternative morphs maintained under frequency- or density-dependent processes) as well as environmental selection regimes, geographic clines in colour-polymorphic species will not necessarily reflect a clear case of local morph-environment matching, as has commonly been portrayed. Indeed, clines in traits that are subject to selection from biotic interactions will not necessarily be similar to clines that simply reflect local adaptation to abiotic conditions or neutral spatial processes (Mallet & Barton, 1989; Nuismer *et al.*, 2000; Gosden *et al.*, 2011; Takahashi *et al.*, 2011; Antoniazza *et al.*, 2014).

The evolutionary dynamics of colour-polymorphic species are often complex, because local morph frequencies and densities within a population can both determine the selective environment faced by individuals (i.e., under frequency- or density-dependent selection, Sinervo et al. 2000; Svensson et al. 2005; Le Rouzic et al. 2015) and, due to altered social interactions, can also affect the expression of morph-specific traits via social plasticity (Johnson 1965; Lancaster et al. 2007). Feedback between selective and formative aspects of the social environment may be positive (i.e., the social environment influences trait expression in the direction of selection; Lancaster *et al.*, 2007) or antagonistic. Positive social feedback can enhance fitness differences between morphs, contributing to increasing clinal variation across the landscape, while negative feedback could instead constrain the potential for adaptive divergence in morph frequencies across environmental gradients. Thus positive social feedback may lead to steeper or different clines in morph frequencies than underlying geographic environmental variation or ecological adaptations would predict alone, and may increase the overall degree of clinal variation, facilitate range shifts, and select against migrants, thereby reducing gene flow across environmental gradients (cf. Mallet and Barton 1989). In contrast, negative social feedback could lead to lower among-site differentiation and an overall narrower species niche breadth than predicted by ecological differences among morphs alone, which in turn might constrain population divergence and the potential for future range shifts.

Our aim in this study was to investigate the relative roles of social (i.e. frequency and density-dependent) and environmental factors for their effects on thermal tolerances of alternative morphotypes, and in turn the consequences of these effects for clinal variation in colour morph frequencies following a recent range expansion. We address these issues in the context of a ~500km geographic cline in morph frequencies at the northern range limit in Sweden of a range-expanding, colour-polymorphic damselfly, *Ischnura elegans* (Hickling et al. 2005; Svensson and Abbott 2005; Gosden et al. 2011; Sánchez-Guillén et al. 2015). This species has been well-investigated in the past in terms of negative frequency-dependent social selection that maintains a female-limited colour polymorphism (Svensson *et al.*, 2005; Gosden & Svensson, 2009; Takahashi *et al.*, 2014; Le Rouzic *et al.*, 2015). We previously identified that recent colonization of higher latitudes in Sweden by *I. elegans* occurred under strong selection

on cold tolerances, imposed by greater climatic variability at recently-colonized, higher latitude sites (Lancaster *et al.*, 2015). Heat tolerance, in contrast, was not under selection during the range expansion (Lancaster *et al.*, 2015). Transcriptomic analysis of heat- and cold-stressed individuals from across this ~500km geographic range confirmed that heat tolerance mechanisms are largely conserved with latitude in this species, while gene expression associated with cold stress is more latitudinally variable (Lancaster *et al.*, 2016).

Here we suggest that changing population morph frequencies at the expanding range margin reflects the joint influence of selection from abiotic environmental conditions in tension against social feedback effects on cold tolerance phenotypes. To investigate this, we assessed thermal tolerances of alternative female morphs from multiple populations in the south of Sweden and near the species' range limit in central Sweden, and identified how these thermal tolerances varied in response to population level social characteristics (population density, observed adult sex ratio, and morph frequencies) and local weather and climates. Here we examine whether any social effects on thermal tolerance exhibited positive feedback or negative feedback with the direction of selection on thermal tolerances (greater strength of selection on cold tolerances in the north; Lancaster *et al.* 2015), which could contribute to clinal variation in morph frequency near the range limit.

Methods

Study system

Ischnura elegans (Vander Linden, 1820; Odonata: Zygoptera: Coenagrionidae) is a widespread and common species throughout Eurasia, and exhibits a female-limited colour polymorphism throughout its range (Askew, 2004; Sánchez-Guillén *et al.*, 2011). Female morphs are distinguishable primarily on the basis of thorax colouration, which can be blue-green (androchrome morph), olive green (infuscans morph), or brown (infuscans-obsolata morph). The thorax colours themselves primarily serve as social signals (Stoks *et al.*, 2001; Hammers & Hans Van Gossum, 2008) and are unlikely to contribute to different thermoregulatory strategies (i.e., there is no 'melanic morph' in this species). Males are uniformly blue or green, and thus the androchrome morph may

represent a male-mimic strategy (Rivera & Sánchez-Guillén, 2007; Abbott & Svensson, 2010), while the other two morphs are distinct from males, exhibit similar mate-avoidance strategies (Stoks *et al.*, 2001), and are collectively called gynochromes.

Energetic and fitness costs of male mating harassment can be quite high in this system, and the polymorphism is thought to be maintained by males targeting common female morphs for mating attempts, thus providing a rare-morph advantage (Fincke, 2004; Gosden & Svensson, 2009; Takahashi *et al.*, 2014). Due to potential behavioral and reproductive differences among morphs reflecting alternative harassment-avoiding tactics (Rivera & Sánchez-Guillén, 2007), or along orthogonal axes of morphic differentiation (Abbott & Svensson, 2010), females also likely exhibit divergent mating, reproductive, and ecological strategies (Stoks *et al.*, 2001; Takahashi & Kawata, 2013). Morph frequencies vary clinally across their geographic range, and previous studies, which did not consider populations near the species' range limits, have reported higher frequencies of androchromes at higher latitudes (Hammers & Hans Van Gossum, 2008; Gosden *et al.*, 2011; Takahashi *et al.*, 2011). Indirect evidence of ecological differences among morphs from population-level data suggests that androchrome frequencies are higher in cooler sites within the core of the species range (Hammers & Hans Van Gossum, 2008) and androchrome frequencies are also higher when populations are at higher densities (Hinnekin, 1987). These data and observations suggest that the different morphs are alternatively adapted to different climate and population density regimes (Hammers & Hans Van Gossum, 2008; Gosden *et al.*, 2011; Takahashi *et al.*, 2011). However, studies addressing putative alternative thermal response phenotypes among the different morphs have been equivocal. Abbott (2013) found that cold temperatures during larval development result in larger sizes of androchromes (but not gynochromes, which are already larger than androchromes in Sweden). In contrast, Bouton *et al.* (2011) found no difference between female morphs in developmental responses to different temperatures experienced during the egg and hatching phase. The fitness consequences of any morphotypic variation in thermally-dependent larval growth rates remains unknown. Here, we examined alternative thermal tolerances of adult individuals, which is the life stage at which the colour polymorphism becomes evident and can therefore contribute to social feedback on others' thermal tolerances.

Like many other insect species, *I. elegans* has been found to be expanding its range northwards in Europe under the current global warming regime. In the UK, *I. elegans* has expanded its range by 143 km in the past 50 years, and has concomitantly increased in population density in the northern part of its range (Hickling *et al.*, 2005). Our sampling of the northern range limit of *I. elegans* in 2013 resulted in discovery of new sites beyond the published range limit and north of all previously reported sightings, suggesting to us that the range expansion is still underway (see also Sánchez-Guillén *et al.* 2015 for projected 2080 range limits for this species). Repeated colonisation events combined with increased selection in new habitats at the range front can increase the possibility for clines to develop during range expansions, in contrast to predicted levels of clinal variation observed in stable populations (Berggren *et al.*, 2012; Antoniazza *et al.*, 2014). Thus the current range expansion offers an ideal scenario to test for social and environmental influences that could cause, support, or reinforce spatial gradients of colour-polymorphic phenotypes as they develop during range expansions.

Field work and thermal trials

Adult damselflies were captured daily throughout the summer flying season from a set of intensively-studied populations representing the range core in southern Sweden in 2012 (Svensson *et al.*, 2005; Le Rouzic *et al.*, 2015), and in 2013 from a similar set of population at the northern range edge in central Sweden. We previously reported on regional variation in thermal tolerances using 25 populations from these regions (Lancaster *et al.* 2015). In the current study, populations with fewer than eight adult females captured were omitted from the analysis. This resulted in $n = 7$ populations at the northern range limit (edge region; latitude = 59.47858 – 59.95853) and $n = 14$ populations in the range core (core region; latitude = 55.60500 – 55.81800). This subsetting allows a more reliable estimate of putative frequency dependent effects on thermal tolerances. We selected 8 as the cutoff because it balanced the resolution and potential accuracy in estimating morph frequencies against loss of data by omitting too many populations from the analysis. However, results were similar when the full dataset of 25 populations was included. Catching bouts were timed to obtain estimates of population density (number of individuals captured per minute of catching effort;

Svensson et al. 2005), and data on sex ratio and morph frequencies was recorded (Table S1). In each region, individual females of each morph were brought back to lab to be weighed (mass recorded in mg) and assessed for cold response phenotypes.

To assess cold response, sexually-mature female damselflies were placed individually in 70cm (diameter) x 30cm (height) containers and provided with ventilation and a water source. 15-25 individuals per day were then placed into a Binder APT.line KB 53 (E3.1) refrigerated incubator (Binder GmbH, Tuttlingen, Germany), with programmable heating- and cooling-rate settings and heat transfer to subjects via convection. After a 25-minute equilibration period at 25° C, individuals were slowly ramped at a rate of 0.6° C min⁻¹ down to 2° C, where they were retained overnight. The end temperature was selected in pilot studies as described in (Lancaster *et al.*, 2015). Following the trial, individuals were returned to room temperature and their recovery time recorded, up to a censor time of 15 minutes. Recovery was scored when individuals flapped their wings in preparation for flight. Because the *infuscans-obsolata* morph is rare in Sweden, this morph was combined with the *infuscans* morph to characterize thermal responses of gynochromes (vs. androchromes), following (Stoks *et al.*, 2001; Sánchez-Guillén *et al.*, 2013) and other previous studies. Time to recovery from chill coma was assessed to estimate morph-differences in the drivers of cold tolerance. Overall, we assessed and analysed chill coma responses for n = 80 mature gynochromes and n = 150 mature androchromes, representing n = 3.71 and n = 4 individual gynochrome females tested per site in the range core and range edge regions respectively, and representing n = 8.93 and n = 3.43 individual androchrome females tested per site in the range core and range edge regions respectively. While we balanced sampling between morphs at each site and in each region as much as possible, the low frequencies of gynochromes in the range core resulted in slightly over-sampling of androchromes there.

Statistical analyses

As previously described in Lancaster et al. (2015), we characterized mean climate within study sites using Bioclim variables (Hijmans *et al.*, 2005), and we characterized weather events leading up to the day of capture using local weather station data from the Swedish Meteorological Institute (www.smhi.se). Population density, sex-ratio, and morph frequencies were obtained from our field data (see

above). To identify factors affecting cold recovery rates for mature females, mixed-effect cox proportional hazard models were implemented in the Coxme package for R (Therneau, 2015). Effects of female morph, female body mass, site-level values for mean annual temperature (Bioclim Bio1) and diurnal temperature range (Bioclim Bio2), more recent temperatures from local weather station data, and population-level values for sex ratio, density, and morph frequencies, latitude, and interaction effects were tested as fixed factors in a single model. Site was included as a random factor. The best model was selected using AIC. We also ran individual models to explain cold tolerances of androchrome and gynochrome females separately, using the same explanatory variables and model selection procedure as in the full model containing all morphs. This latter approach provides greater resolution of predictive environmental effects within each morph, but results of the separate models cannot be used to infer significant differences between the morphs. Plotting of thermal response phenotypes was done in the coxph package for R (Figure 2).

We estimated among-region differences in social and environmental factors in single-factor anovas using population-level data on climate, density, sex ratio, and morph frequencies, using populations as replicates. Regional differences in mass were assessed using individual-level data, and including region as a fixed factor and population as a random factor in a mixed model. To identify correlations between different social and environmental predictor variables (i.e., correlations of density with climate) among sites, we used partial Mantel tests to control for effects of spatial relationships among populations. Analyses were performed in R v3.3.0 (R Core development Team, 2012), with the lme4 and LmerTest packages (Bates *et al.*, 2014; Kuznetsova *et al.*, 2014) used for mixed models and the Ecodist package (Goslee & Urban, 2007) for Mantel tests. Reported error terms are standard errors. The ggmap package for R (Kahle & Wickham, 2013) was used to create figure 1.

Results

Factors affecting thermal tolerance of the morphs

Recovery rates from the cold-ramp experiment were best predicted by a model which included an effect of the female's morphotype (androchromes exhibited better cold tolerance; Table 1A, Figure 2A), morphotype x mass, minimum temperature over the past 3 days and the frequencies of female morphs at the site (Table 1A). Thus in the

full model, cold hardiness was overall improved by recent cold weather events, and all mature females were generally more cold tolerant at sites characterised by higher gynochrome frequencies.

Modeling cold tolerance separately within each female morphotype, we find that androchrome chill coma recovery was best predicted by a model that included only minimum temperatures over the past 3 days (Table 1B, Fig. 2C; androchrome cold tolerance was improved after recent exposure to cold weather). In contrast, gynochrome chill coma recovery was best explained by a model that included the female's mass and local morph frequencies at her site of capture (Table 1C, Figure 2C,D). Heavier gynochromes recovered more quickly from a cold challenge than lighter gynochromes, and an increasing frequency of gynochromes in the population was associated with quicker recovery times for gynochromes.

Latitudinal clines in ecological and social predictors of cold tolerance within each morph.

Using a model including a random factor to account for populations sharing weather station data, we found that minimum temperatures over the past 3 days prior to capture were lower at the range edge than in the core (effect of region on the average recent minimum temperatures for individuals for each population = 1.28 ± 0.51 , $t = 2.51$, $P = 0.02$; Figure 1). Population-level gynochrome frequency also differed between sampling regions (effect of region on morph frequency = 0.37 ± 0.07 , $t = 5.10$, $P < 0.0001$; Figure 1; gynochrome frequencies were higher at the range edge). Body mass, which also explained gynochrome cold tolerance (more massive = more cold tolerant), decreased towards the range edge in females of both morphs (effect of region on body mass of mature females = -5.47 ± 1.43 , $t = -3.81$, $P = 0.001$; Figure 3), and this effect did not differ by morph. We also found that gynochromes were heavier than androchromes at all latitudes (effect of morph on mature female body mass = 2.42 ± 0.71 , $t = 3.41$, $P = 0.007$; Figure 3). Due to differing but complementary mechanisms affecting variation in cold tolerance variation within morphs, all females exhibited increased cold tolerance at the range edge in comparison to the core (effect of region on chill coma recovery time = 0.42 ± 0.66 , $z = 2.32$, $P = 0.02$).

Relationships among predictor variables:

Correcting for spatial distances among sites, we found that the proportion of gynochromes at a site was negatively correlated with mean annual temperature (Mantel $r = -0.28$, $P = 0.01$; Figure 3), but morph frequencies are uncorrelated with changes in recent minimum temperatures leading up to capture dates across our study sites (Mantel $r = 0.07$, $P = 0.24$). In contrast to previous findings (Hinneking 1987), morph frequencies did not correlate with population density (Mantel $r = -0.14$, $P = 0.12$) or with sex ratio (Mantel $r = 0.11$, $P = 0.93$). Among-population variation in female mass did not correlate with population density, sex ratio, morph frequencies, or climate variables in a spatial analysis.

Discussion

Clinal variation in morph frequencies across environmentally heterogeneous landscapes results from complex processes, because both phenotypic expression and fitnesses of each morph respond to social processes such as frequency-dependence, as well as to non-social, environmental factors and neutral processes. We find that *I. elegans* morph frequencies exhibit a unique latitudinal cline in morph frequencies near their northern range limit that corresponds to recent range expansion history and climatic differences among sites. Specifically, gynochromes are more common in cooler and more recently colonised sites along the northern range margin, while androchrome frequencies are higher in warmer sites which are closer to the population core, although still relatively far north in reference to the mid-point of the geographic range. This result suggests that events during the recent range expansion may have acted to counteract the continent-wide latitudinal cline across the range core for this species, in which androchrome frequencies increase between Southern Europe and Southern Sweden (Gosden et al. 2011). We also found pronounced differences in how underlying social and climatic environmental variation shaped thermal tolerances within each morph.

Both androchrome and gynochrome cold tolerances responded to ecological and social environments in ways that appeared to facilitate better cold tolerance at higher latitudes. In a model considering the general drivers of variation in cold tolerance across all females, it was apparent that spatially-varying social (gynochrome frequencies) and climatic effects (recent exposure to cold) had beneficial consequences

irrespective of morph. However, these individual environmental factors did not equally explain variation in cold tolerance observed within each morphotype. Variation in androchrome cold tolerance was best explained only by variation in recent weather events, such that individuals of this morph produce more cold-tolerant phenotypes under conditions of recent cold weather events. This is a classic example of a beneficial acclimation strategy, which is commonly reported in invertebrates (Gunderson & Stillman, 2015). This phenotypic shift in direct response to thermal conditions allows androchromes to cope with intensifying climatic variability at high latitudes. However, androchromes might be unable to reach high frequency at the northernmost range limit where weather conditions are more variable, and where cold weather events are more frequent than in their historic conditions in the range core. Our findings corroborate a recent meta-analysis suggesting that adaptive plasticity in thermal tolerance is generally insufficient to prepare species for novel sources of thermal stress (e.g., during a range expansion or in response to climatic warming; Gunderson and Stillman 2015).

In contrast to androchromes, variation in cold tolerance within gynochromes was not well explained by variation in recent environmental thermal conditions via acclimation, but was strongly influenced by social factors. Cold tolerance variation within gynochromes was strongly affected by the frequency of gynochromes in the population, with gynochromes exhibiting higher cold tolerances when captured from sites characterised by high gynochrome frequencies. The mechanism facilitating this effect is unknown. One possibility is that cold tolerance is directly affected with both individual personality and the levels of social stress that the individual has recently experienced. A similar mechanism recently been partially reported for salmonid fish, in which subordinate individuals exhibit overexpression of stress-inducible chaperone proteins in comparison to dominant fish, in response to social stress and prior to any thermal challenges (Currie *et al.*, 2010; LeBlanc *et al.*, 2011). Thus social stress may have a pleiotropic effect on thermal stress-response enzyme pathways that mimics cold weather acclimation. In *I. elegans*, gynochromes innately exhibit lower levels of behavioural dominance than their androchrome counterparts (Stoks *et al.*, 2001). Due to their phenotypic differentiation from males, gynochromes can also experience higher harassment rates from males, especially at high frequencies (Fincke 2004), a severe source of social stress. If social stress provides a cold-tolerance priming mechanism in *I. elegans*, then our results suggest that higher harassment rates of gynochromes when at

high frequencies may indirectly enhance their cold tolerances. Furthermore, the larger body mass of gynochromes (in comparison to androchromes) is likely to have initially provided a cold-tolerance advantage (e.g., increased flight muscle mass may increase the capacity for shivering thermogenesis), and this body size advantage in conferring cold tolerance (see results) could later have been amplified by social feedback.

Of the two, distinct, underlying causes of latitudinal variation in cold tolerance in each morph, one involving social feedback and the other based on recent environmental temperature variation, social effects had stronger explanatory power for the observed cline in morph frequencies near and at the expanding range margin. Thus putative selective drivers of spatial variation in morph frequencies in this system do not simply reflect straightforward environment-dependent matching of morph-specific variation in cold resistance to local abiotic conditions. In such a straightforward scenario of local adaptation to abiotic environmental conditions, androchromes, which were better able to respond to underlying climatic variation, should have been observed to be more frequent at the expanding range edge. Instead and rather unexpectedly, gynochromes increase in frequency at northernmost sites with cooler and harsher climates near the poleward expansion front for this species. These high gynochrome frequencies towards the range limit might result from or be reinforced by a positive frequency-dependent thermal tolerance advantage to gynochromes there (Figure 2A,D, 3).

Increasing frequencies of gynochromes towards the range limit likely results in part from a slight thermal tolerance advantage that these social environments confer. However, effects of genetic drift and morph- and sex- differences in larval growth processes in response to novel climates (Gosden et al, 2011, Abbott 2013) might also contribute to the observed cline reversal. These combined effects during range expansions may thus reverse or counteract the larger-scale geographic cline across Europe with increasing frequencies of androchromes at higher latitudes in *I. elegans* (Hammers & Hans Van Gossum, 2008; Gosden *et al.*, 2011). Recent work at the *I. elegans* poleward range expansion front in Great Britain also suggests that gynochromes increase in frequency towards cooler sites within the recently-colonised region (Lancaster and Fitt, unpublished data). This provides additional support that the continent-wide cline is typically reversed at the poleward expansion front in this species. Note also that the positive frequency-dependent effects on gynochrome cold

tolerance at the range limits counteracts the typical negative frequency-dependent selection that maintains all alternative morphs in populations within the range core (Le Rouzic *et al.*, 2015). Together these results suggest that the demographic processes underpinning local morph frequency maintenance and large-scale clinal variation in morph frequencies in the established portion of the species range might differ substantially from the demographic processes occurring at the range margin. At the range margin, local populations will encounter novel environmental variation and experience demographic expansion. These differences between range core and range limit populations may contribute to selection for social benefits that are unique to range shifting populations. Such social benefits may contribute to the observed, unexpected clinal reversal during colonisation at the expanding range limit.

Conclusions

Ecological differences among morphs have previously been predicted to influence geographic clines in morph frequencies within colour-polymorphic species (West-Eberhard, 1986; Corl *et al.*, 2010; Berggren *et al.*, 2012). However, we find that a morphotypic cline along a thermal gradient was more strongly related to morph-specific differences in social responses than ecological responses, possibly because social interaction effects can be amplified by positive frequency-dependent feedback, while ecological response traits cannot. Our results suggest that whenever phenotypes or fitnesses depend on population-level processes (i.e., under frequency- or density-dependent selection, or effects of conspecifics on trait expression), social feedback can reverse or reinforce clines along ecological gradients, particularly during range shifts and under novel sources of stress such as climate change.

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Table 1. Fixed effects in the best models explaining drivers of cold stress responses (recovery time from cold-ramping experiments), for mature female *Ischnura elegans* individuals in Sweden.

a) All females :

<i>Effect:</i>	<i>Estimate:</i>	<i>z:</i>	<i>P:</i>
Morph (affect of androchrome)	-3.86 ± 1.37	-2.81	0.005
Morph x body mass	-0.07 ± 0.03	-2.19	0.03
Three-day minimum temperature	0.09 ± 0.05	2.07	0.03
Local gynochrome frequency	-1.20 ± 0.63	-1.90	0.05

b) Androchrome females only:

<i>Effect:</i>	<i>Estimate:</i>	<i>z:</i>	<i>P:</i>
Three-day minimum temperature	0.11 ± 0.05	2.44	0.01

c) Gynochromes] females only:

<i>Effect:</i>	<i>Estimate:</i>	<i>z:</i>	<i>P:</i>
Body mass	-0.07 ± 0.03	-2.26	0.02
Local gynochrome frequency	-1.66 ± 0.85	-1.95	0.05

Supplementary files:

Table S1: Morph frequencies, population densities, and sex ratios of adult *Ischnura elegans* at study sites.

Figure legends:

Figure 1: Location of study populations within and beyond the published range limit. A. Towards the northern range expansion front, thermal tolerances of androchromes respond to recent weather events (more orange locales = cooler minimum temperatures over the 3-day window prior to individual capture days), while gynochromes improve thermal tolerances via social feedback (size of circles

corresponds to proportions of gynochromes at study sites). Previously-published range limit for *I. elegans* is from Dijkstra and Lewington (2006). B. Ternary plot showing morph frequencies at individual study sites at the range limit (red) and in the range core (blue). See Table S1 for raw data.

Figure 2: Environmental factors affecting thermal tolerance of different female morphs, where thermal tolerance is estimated as the rate of recovery from a low-temperature physiological challenge. A. Variation in female cold tolerance by morph and region. B. Effect of minimum temperatures over the previous 3 days prior to capture on androchrome cold tolerances (a beneficial acclimation effect). C. Effect of body mass on cold tolerance of gynochromes. D. Effect of gynochrome frequency on gynochrome cold tolerance (beneficial social feedback). *For visualization purposes only*, continuous predictor variables (panels B-D) were artificially bisected into two categories (above- vs. below-average values for predictor variables), and mean and confidence intervals of thermal tolerances associated with these categories are depicted. See Table 1 for statistical results.

Figure 3: Relationships among predictor variables (solid lines = positive relationships, dashed lines = negative relationships, curved arrows = relationships among predictor variables). A. Androchromes exhibited adaptive plasticity in cold tolerance in response to relevant cold weather events, which were more common at the range edge. B. Gynochromes recovered better from cold challenge if they were more massive or if they were from a population with a higher frequency of gynochromes, which were also more common at the range edge. Positive feedback between gynochrome chill coma recovery and frequency of gynochromes in the population putatively reinforces higher frequencies of gynochromes in cooler climates at the northern range limit.

Figure 1:

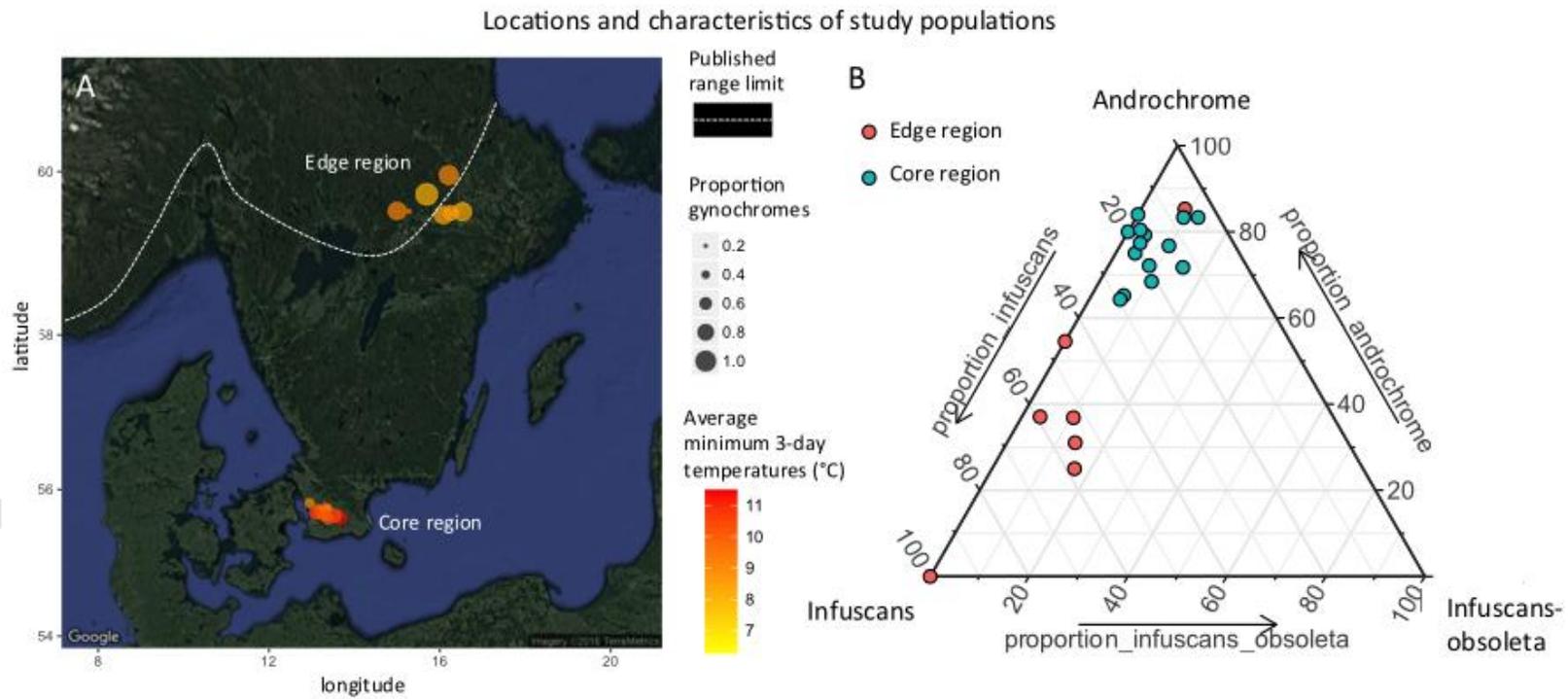


Figure 2:

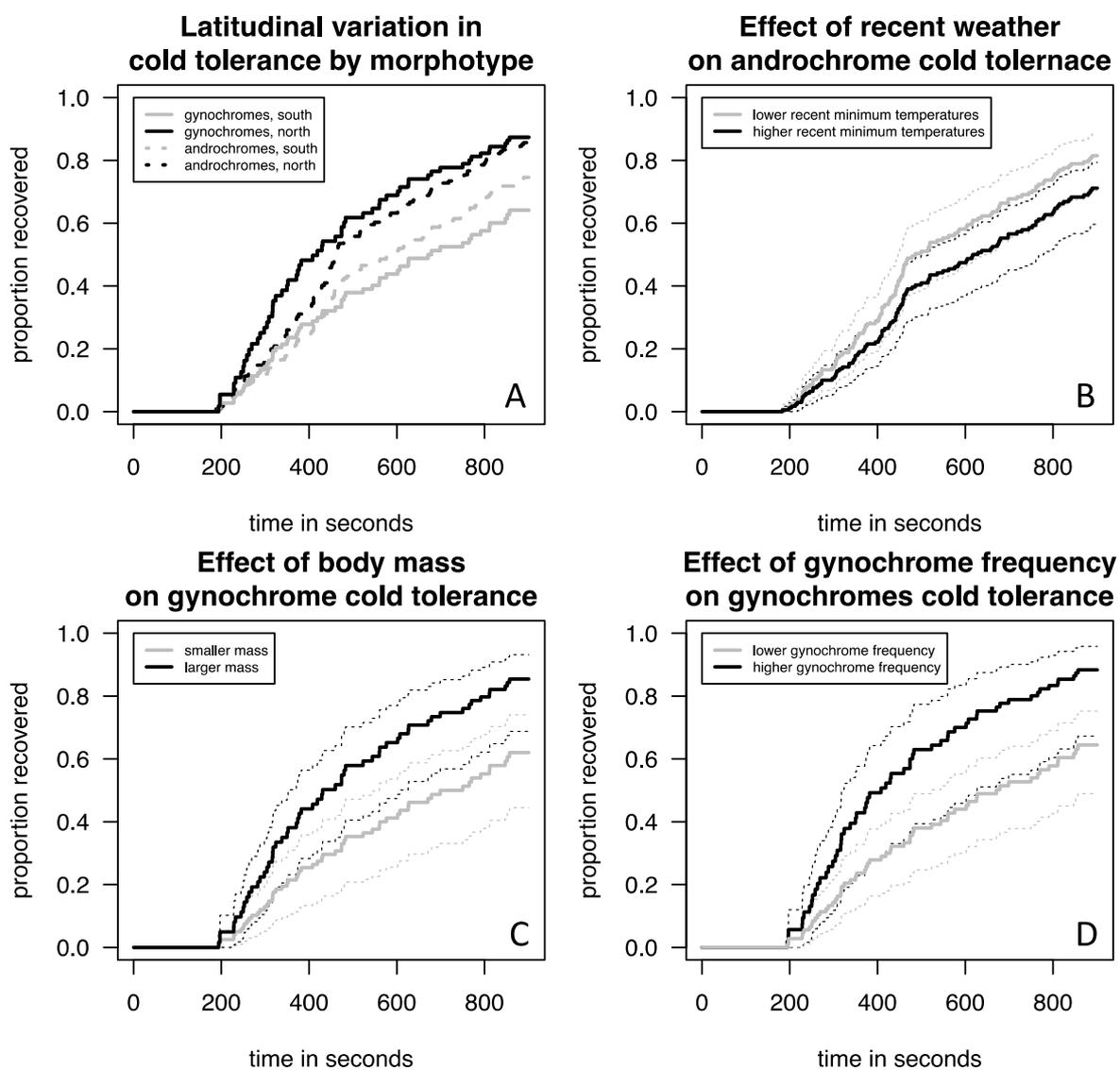


Figure 3:

