LETTER

Predicting evolutionary responses to interspecific interference in the wild

Gregory F. Grether,[1]* Jonathan P. Drury,[2] Kenichi W. Okamoto,[3] Shawn McEachin[1] and Christopher N. Anderson[4]

[1]Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA, USA

[2]Department of Biosciences, Durham University, Durham, UK

[3]Department of Biology, University of St. Thomas, St. Paul, MN, USA

[4]Department of Biological Sciences, Dominican University, River Forest, IL, USA

*Correspondence: E-mail: ggrether@g.ucla.edu

Abstract

Many interspecifically territorial species interfere with each other reproductively, and in some cases, aggression towards heterospecifics may be an adaptive response to interspecific mate competition. This hypothesis was recently formalised in an agonistic character displacement (ACD) model which predicts that species should evolve to defend territories against heterospecific rivals above a threshold level of reproductive interference. To test this prediction, we parameterised the model with field estimates of reproductive interference for 32 sympatric damselfly populations and ran evolutionary simulations. Asymmetries in reproductive interference made the outcome inherently unpredictable in some cases, but 80% of the model's stable outcomes matched levels of heterospecific aggression in the field, significantly exceeding chance expectations. In addition to bolstering the evidence for ACD, this paper introduces a new, predictive approach to testing character displacement theory that, if applied to other systems, could help in resolving long-standing questions about the importance of character displacement processes in nature.

INTRODUCTION

Character displacement theory classically pertains to two types of mutually negative interactions between coexisting species: exploitative competition arising from overlap between species in resource use [ecological character displacement (ECD)] and reproductive interference arising from incomplete mating isolation (reproductive character displacement [RCD]; Brown & Wilson 1956; Pfennig & Pfennig 2012). This theory has recently been expanded to include another common type of mutually negative species interaction: interspecific interference competition (Grether *et al.* 2009, 2013, 2017). While interference competition also occurs in plants and microbes, so far theory developed in this area has focused on animals, where interference competition usually takes the form of agonistic interactions, such as aggression, dominance and territoriality; hence this addition to character displacement theory is known as agonistic character displacement (ACD).

Interference competition can be a costly interaction that species evolve to avoid (divergent ACD) or the product of evolved responses to interspecific competition for mates or other resources (convergent ACD). Although these two aspects of interference competition were studied separately for decades and not regarded as forms of character displacement (e.g., Lorenz 1962; Cody 1969; Case & Gilpin 1974; Gill 1974; Diamond 1982; Hairston 1983; Nishikawa 1987; Peiman & Robinson 2007), they have been unified in ACD models where the value of one continuous parameter – the level of resource overlap or mate competition – can determine whether the species diverge or converge in competitor recognition and the traits upon which competitor recognition is based

(e.g., territorial song, coloration, pheromones; Grether *et al.* 2009; Drury *et al.* 2015). Convergence in territorial signals (i.e., convergent ACD) can result in interspecific territoriality, which is a form of resource partitioning that reduces interspecific exploitative competition (Cody 1969; Grether *et al.* 2009).

Traditionally, character displacement is studied by documenting a geographic pattern of trait variation consistent with character displacement (e.g., divergence or convergence in sympatry) and then attempting to rule out alternative explanations for the pattern, such as chance, genetic drift, hybridisation and species sorting, while also testing assumptions of the character displacement hypothesis (Schluter 2000; Pfennig & Pfennig 2012). This approach has been applied to several putative examples of both convergent and divergent ACD (Grether *et al.* 2009, 2013), and some new case studies are particularly compelling (e.g., darters, Moran & Fuller 2018a,b; antbirds, Tobias & Seddon 2009, Kirschel *et al.* 2019; nightingales, Reif *et al.* 2015; Souriau *et al.* 2018; singing mice, Pasch *et al.* 2017; damselflies, Anderson & Grether 2010a,b; Drury & Grether 2014). However, while the traditional approach can provide strong evidence that character displacement has occurred in particular cases, it provides little information about its prevalence or predictability (Germain *et al.* 2018).

Here we pioneer a predictive approach to testing character displacement theory that is practical at the scale of small clades (e.g., genera). It involves developing a character displacement model based on the focal clade, simulating conditions at the time of secondary contact for multiple species pairs, and then comparing the model's evolutionary predictions to outcomes observed in sympatry. As natural

populations are subject to selection in other contexts, as well as genetic drift and gene flow, we would not expect any character displacement model to predict all outcomes precisely, but a useful model should outperform chance expectations. We apply this approach to rubyspot damselflies (*Hetaerina* spp.), a genus that has been inferred to exhibit divergent ACD in some cases (Anderson & Grether 2010a,b; Drury & Grether 2014) and convergent ACD in others (Drury *et al.* 2015). Essentially, we ask whether ACD theory can correctly predict which sympatric populations fall into which of these two categories.

The hypothesised difference between divergent and convergent damselfly populations is the level of reproductive interference – more specifically, the extent of local mate competition between species caused by males attempting to mate with heterospecific females (Drury *et al.* 2015). Although interspecies pairs break up prior to copulation, males can clasp and fly in tandem with heterospecific females, potentially resulting in interspecific mate competition within male territories (Drury *et al.* 2015). The ability of males to distinguish between conspecific and heterospecific females varies considerably among species pairs (Drury *et al.* 2015) but is not enhanced in sympatry compared to allopatry (Drury *et al.* 2019). Thus, current levels of reproductive interference are probably representative of the levels that occurred at the time of secondary contact.

An ACD model based on the life history and behaviour of this system predicted that sympatric populations would diverge in competitor recognition traits until interspecific territoriality is eliminated if the reduction d in a male's mating success caused by sharing a territory with one heterospecific male is below a threshold, and to converge in the same traits until interspecific territoriality is established if d is above the threshold (Drury *et al.* 2015). Drury *et al.* (2015) carried out territory intrusion experiments on multiple sympatric populations and obtained results consistent with the model's predictions: territory holders were more aggressive to heterospecific male intruders at sites where heterospecific males were more likely to attempt to mate with females of the territory holder's species. Drury *et al.* (2015) were unable to test the model directly, however, because they did not have empirical estimates of d. Furthermore, their model assumed that d and population density are symmetrical between sympatric species, and the consequences of relaxing these assumptions were unknown.

Here we advance ACD theory by exploring the consequences of asymmetries between sympatric species in the cost of sharing space with heterospecifics. We further test whether ACD theory can predict how specific populations have evolved in response to each other. To this end, we modified the model of Drury *et al.* (2015) to allow for asymmetries in d and population density, obtained field estimates of these parameters for 16 pairs of sympatric *Hetaerina* populations, and then used the model to simulate secondary contact between populations with the observed values of d and population density, tracking their evolution for 9000 generations. The evolvable traits in the model include a male trait upon which competitor recognition is based (z) and two 'neural' traits (μ and σ) that together govern the competitor

recognition function (Grether *et al.* 2009). When males meet in the model, whether they respond aggressively to each other and fight over the territory depends on their respective values of these three traits. Over time, the fitness consequences of the interactions cause the traits to evolve. If the species' traits evolve away from each other, reducing interspecific aggression, divergence results. If the species' traits evolve towards each other, increasing interspecific aggression, convergence results. If chasing occurs (i.e., one species' traits evolve towards the other's and the other species' traits evolve away), a stable outcome of divergence or convergence might eventually be reached, or the outcome might be unstable. We calculated the mean ratio of heterospecific/conspecific aggression over the last 1000 years of each evolutionary simulation and compared the model's predictions to the observed mean levels of aggression of territory holders towards male intruders in the field.

To the best of our knowledge, this is the first time a character displacement model has been used to generate predictions for particular species pairs in the wild, and thus a milestone for ACD and character displacement research in general.

MATERIALS AND METHODS

Study populations

Hetaerina is a genus of 38 species patchily distributed in river drainages on the Atlantic and Pacific slopes of the Americas (Garrison 1990). For this study, we collected data at 13 sites with two or more sympatric species; two sites in the U.S., five in Mexico and six in Costa Rica (10 different species; 13 different species pairs; Table S1). Fieldwork was conducted between March 2006 and July 2017.

Field estimates of reproductive interference

The cost (d) of sharing space with heterospecifics (see Introduction) cannot be measured directly because male *Hetaerina* do not usually share territories. We therefore used a Monte Carlo simulation to estimate what the reduction in a male's mating success would be if heterospecific males shared territories. We assumed that clasping probabilities in sympatry can be used to simulate conditions at the time of secondary contact, because clasping probabilities do not differ between population in sympatry and allopatry, and most *Hetaerina* populations are strongly differentiated genetically (Drury *et al.* 2019). Nevertheless, clasping probabilities alone are insufficient for estimating d. In principle, sharing a territory with a heterospecific male could enhance a male's mating success if interspecific tandem pairs usually break up close to the point of clasping. Other variables that affect d include the latencies with which males of the two species clasp females, the probability of males following interspecific tandem pairs, the distances males transport heterospecific females before releasing them, and the probabilities of perched males clasping conspecific females at different distances. We therefore carried out field experiments to obtain sampling distributions for each of these variables, as described below. For clarity, we describe a single experimental trial of each type, but the sampling

distributions were based on multiple trials (for sample sizes, see Table S2):

(1) Clasping probabilities and latencies. Method: A live, tethered female was flown into the territory of a male, within 0.5 m of the male's perch, for 5 s. We recorded whether the male clasped the female and, if so, the time from the start of the trial to when clasping occurred.

(2) The probability of a male following an interspecific tandem pair and therefore being closer to the point of release, and more likely to re-clasp the female, than he would be if he remained perched. Method: A female was attached to a heterospecific male with transparent thread, the pair was tethered and flown through the territory of a male of the female's species, and whether the territory holder followed the pair was recorded.

(3) Heterospecific release distance. Method: A female was released from a wire and mesh cage at the end of a pole, directly below a perched heterospecific male. If the male clasped or attempted to clasp the female, we recorded the distance from the male's initial perch to his last point of contact with the female.

(4) The probability of a female being clasped by a conspecific male after being released by a heterospecific male at a given distance from the conspecific male's perch. Method: Same as (1) except that females were also flown at distances of 1–2 m and 2–3 m from the perches of conspecific males. We fit an exponential decay function to the midpoints of the three zones (0.5, 1.5 and 2.5 m) to estimate the probability of a male clasping a conspecific female at any distance between 0.5 and 3 m (beyond 3 m, the probability of clasping is negligible).

We prioritised obtaining population-specific clasping probabilities and carried out the other field experiments as the availability of females and time constraints permitted. We pooled data from experiment (2) across sites to obtain a single estimate of the probability of a male following an interspecific tandem pair (0.264, $N = 155$ trials). From experiments (3) and (4), we used population-specific estimates if we reached a sample size ≥ 20 for the population, species-specific estimates if we reached a sample size ≥ 20 for the species, and pooled estimates across species and sites otherwise.

In the Monte Carlo simulation (Fig. S1), a female arrives on a territory shared by two males and the empirical distributions of clasping probabilities and latencies are sampled to determine which male clasps her first. If the heterospecific male clasps her first, the conspecific male follows the pair with some probability and has a high probability of clasping the female when she is released. If the conspecific male does not follow the pair, the empirical distribution of heterospecific release distances is sampled, and the conspecific male re-clasps her with the empirically measured probability of clasping at that distance. Within 10^5 iterations, the simulation yields a stable estimate of P_{ij}, the probability of a male of species i clasping a conspecific female if the territory is shared with one male of species j. We ran the simulation 10 times for each pair of populations and used the mean values of P_{ij} to calculate $d_{ij} = (P_i - P_{ij})/P_i$, where P_i is the empirically measured probability of a male of species i clasping a conspecific female in

the absence of interference (Table S4). A d value of 0.5 would mean that sharing a territory with one heterospecific male reduces a male's expected mating success by 50%. If the presence of a heterospecific male resulted in local mate enhancement instead of local mate competition, d would be negative. For simulation code and sampling distributions, see Supporting Information.

ACD model with asymmetrical reproductive interference

We used a mechanistic evolutionary model to predict levels of heterospecific aggression for sympatric populations with the observed field estimates of d. The model is individual-based (DeAngelis & Mooij 2005) and the loci and alleles underlying the evolvable traits are tracked explicitly (Okamoto & Grether 2013). We modelled diploid, sexually reproducing populations without overlapping generations, as in *Hetaerina*. The evolvable traits are the central location (μ) and width (σ) of the male competitor recognition function and the male trait (z) upon which competitor recognition is based (Okamoto & Grether 2013). Mutations occur with probability 10^{-4} at each locus and new allelic values are drawn from a Gaussian distribution with a standard deviation of 10% of the mean initial allelic value (Okamoto & Grether 2013).

On each simulated day during the breeding season (90 days), mature males that do not already occupy a territory enter a territory at random. If the territory is occupied by another male, three outcomes are possible: mutual recognition as competitors, one-sided recognition and mutual non-recognition. Which outcome occurs is a stochastic function of the males' respective values of z, μ and σ, according to probabilities $\exp(-(z_i-\mu_r)^2/2\sigma_r^2)$ and $\exp(-(z_r-\mu_i)^2/2\sigma_i^2)$, where subscripts r and i represent resident and intruder respectively (Okamoto & Grether 2013). Mutual and one-sided recognition result in territorial fights, the outcome of which is determined by a probability function based on the males' respective energy reserves for fighting (Okamoto & Grether 2013). The winner of the fight occupies the territory, the loser leaves, and both males suffer a reduction in energy reserves (energy reserves are replenished through foraging according to an empirical age-dependent function) (Okamoto & Grether 2013). If mutual non-recognition occurs, the males share the territory and suffer no loss in energy reserves. Once territory allocation is complete, the probability that a given male mates with a given female depends on whether they are conspecifics, whether he occupies a territory, and for territory holders, the number and species of any other males on the territory (e.g., the presence of one heterospecific male reduces a male's probability of mating by d). Female mating rates, the proportion of females that mate with territory residents, as well as variables governing the underlying population dynamics are based on empirical data and equations in the appendices of Okamoto & Grether (2013).

In *Hetaerina*, competitor recognition is based on male coloration but female mate recognition is not (Grether 1996; Drury & Grether 2014). Accordingly, the ACD model assumes that a female's willingness to mate is based on traits other than z that vary among species (e.g., the size and shape of male claspers; Garrison 1990). Because female mate recognition occurs post-clasping in *Hetaerina* and is not based on

the same male traits as male competitor recognition (Drury & Grether 2014), we did not include evolvable traits for female mate recognition in the model. If female pre-clasping mate recognition were evolvable (with females controlling whether they are clasped), then all populations would evolve towards zero reproductive interference and zero interspecific aggression (Okamoto & Grether 2013). Male mate recognition was also assumed not to evolve, based on evidence that reproductive interference does not affect the evolution of male mate recognition in *Hetaerina* (Drury *et al.* 2019).

Evolutionary simulations

We simulated secondary contact between species that initially were 0.1 standard deviation units apart in z and μ, with σ set to an initial value of 1 standard deviation unit. This initial divergence results in a heterospecific aggression (HA) ratio of approximately 0.8, meaning that the probability of a male responding aggressively to a heterospecific male is about 80% of the probability of responding aggressively to a conspecific male. However, as shown previously, the initial level of divergence does not affect the final outcome (Drury *et al.* 2015). Secondary contact occurred after a 1000 generation allopatric burn-in period, during which the populations drifted somewhat in their mean values of z, μ and σ. The carrying capacities of the larger populations were fixed at 8000 and the carrying capacity of the smaller populations were adjusted to yield the relative population densities observed in the field (based on the number of individually marked males). Based on our repeated visits to the same sites in different years, the relative population densities appear to be stable. Field estimates of the reproductive interference parameter d were obtained as described above.

For each population pair, we ran 10 replicate simulations of 10^4 generations. The mean trait values, population sizes and numbers of encounters and recognition events were recorded in each generation, and from those records, we calculated the HA ratio for each population in each generation (e.g., see Fig. S2). To generate predictions against which to compare the HA ratios observed in the field, and to evaluate the stability of the predictions, we calculated the harmonic mean and variance of the HA ratio over the last 1000 years of each simulation, and then calculated the mean variance and the variance and mean of the harmonic means across the 10 replicates.

By visually inspecting the trait plots (e.g., Figs S2–S7), we classified the simulated evolutionary responses as 'diverge' or 'converge' and the outcome of the species interaction as 'convergence', 'divergence' or 'chasing'. Chasing occurred when one population converged and the other population diverged. Replicate simulation runs were very consistent, so there was no difficulty in scoring the predicted evolutionary responses of the simulated populations. We classified cases with an HA ratio variance across replicates of less than 0.02 as 'stable' and cases with greater variability as 'unstable'.

Territorial aggression

To test the model's predictions, we measured territorial aggression in the field. We marked males individually,

identified territory holders based on observations of site fidelity and defence, and presented them with live, flying, tethered male intruders, as in Anderson & Grether (2010b). Each territory holder was presented with conspecific and heterospecific intruders, with the presentation order balanced across males of each species at each site. Trials were 2 min long with an intertrial interval of > 5 min. From audio recordings of the behaviours observed, we measured the proportion of time spent chasing and the rate of physical midair attacks. Damselflies are sensitive to time of day and weather conditions, and we aimed to test males when they were actively defending their territories. Cases in which a male did not chase either intruder were thus excluded from the analysis. Individual males were only retested if they failed to respond in a previous test. Population means, confidence intervals and sample sizes are shown in Table S3. We used population means to calculate HA ratios, defined as the mean level of aggression towards heterospecific males divided by the mean level of aggression towards conspecific males. For each pair of sympatric populations, we calculated one HA ratio based on physical attack rates and another based on chase durations for comparison with the ACD model's predictions.

Data analysis

The ACD model's predicted HA ratios were strongly clustered around 0 and 1, and the observed HA ratios were also bimodal, and thus the data were not suitable for statistical methods that assume normality of residuals. We therefore used Spearman rank correlations to measure the strength of association between the predicted HA ratio and d and between the observed and predicted HA ratios (in all cases, $N = 32$ populations). To evaluate the model's accuracy in predicting whether field populations have diverged or converged in competitor recognition, we dichotomised the HA ratios based on a threshold of 0.5 (i.e., where aggression towards heterospecific males is 50% as high as aggression towards conspecific males). To compute the probability of the results under the null hypothesis that observed and predicted outcomes are independent, we used Fisher's exact test.

To evaluate whether the observed or predicted HA ratios are correlated with patristic (phylogenetic) distance between species, we used a randomisation approach that circumvents potential non-independence caused by the data structure (i.e., each pair of populations has two HA ratios but only one patristic distance). One population in each pair was dropped at random and the Spearman correlation coefficient (r_s) was computed using the remaining 16 data points. This was repeated 10^4 times, and the resulting mean r_s was compared to Spearman correlation critical values for $N = 16$. Patristic distances were obtained from the phylogeny of Drury *et al.* (2019).

RESULTS

Model predictions

We found considerable variation in the level of reproductive interference between populations but no cases of local mate

enhancement or facilitation (sensu Bruno *et al.* 2003). The *d* estimates ranged from 0 to 0.502 (mean = 0.194, SD = 0.134, *N* = 32; Table S4). The ACD model predicted divergence when the *d* parameter of both populations was < 0.23, convergence when the *d* parameter of both populations was 0.23 or higher, and chasing when the *d* parameters of the two populations fell on different sides of this threshold (Fig. 1; Table S4; for examples of each outcome, see Figs S2–S7). Asymmetries in population size influenced rates of divergence and convergence (e.g., Figs S2–S5) and the stability of the chasing outcomes (cf. Figs S6 and S7). All 22 populations with predicted outcomes of divergence or convergence, and three of the 10 populations with chasing as the predicted outcome, had stable HA ratio predictions (Fig. 2). In one of the chasing cases, different replicates predicted alternative stable HA ratios (Fig. 2). Across populations, the predicted mean HA ratio was strongly positively correlated with *d* (Fig. 3; r_s = 0.76, *P* < 0.0001, *N* = 32).

Comparing aggression in the wild to model predictions

Observed and predicted HA ratios were strongly positively correlated across populations (Fig. 4, Table S5; observed attack rate ratio, r_s = 0.60, *N* = 32, *P* = 0.0003; observed chase duration ratio, r_s = 0.65, *N* = 32, *P* < 0.0001). The model correctly predicted the categorical outcome for 24 of the 32 populations (Fisher's exact test, *P* = 0.0028) and 20 of the 25 population with stable predictions (*P* = 0.0036; Table 1).

Among the populations with stable but incorrect predictions, four had high observed HA ratios but were predicted to

have low HA ratios, and one population with a low observed HA ratio was predicted to have a high HA ratio (Table 1). The latter population has a *d* estimate just above the model's divergence/convergence threshold (0.23). If the true level of reproductive interference was slightly lower, the predicted outcome would be chasing. Thus, the only clear exceptions to the model's predictions are cases where HA in the field is higher than predicted.

Neither observed nor predicted HA ratios were correlated with patristic distance between species (predicted HA ratio, mean r_s = 0.06, *N* = 16, *P* = 0.82; observed attack rate ratio, mean r_s = 0.32, *N* = 16, *P* = 0.22; observed chase duration ratio, mean r_s = 0.16, *N* = 16, *P* = 0.55).

DISCUSSION

Many interspecifically territorial species also interfere with each other reproductively (see Table S1 in Drury *et al.* 2015), and the hypothesis that aggression towards heterospecifics is an evolved response to interspecific mate competition has been proposed multiple times (e.g., Payne, 1980; Baker, 1991; Sedlacek, Cikanova, & Fuchs, 2006; Reichert & Gerhardt, 2014). This hypothesis was formalised in an ACD model that predicts the level of reproductive interference at which species should converge in competitor recognition and defend interspecific territories (Drury *et al.* 2015). To test the model, we obtained field estimates of reproductive interference for 32 sympatric damselfly populations and ran model simulations with those estimates. In seven cases, asymmetries in reproductive interference made the outcome inherently unpredictable, which is an interesting and seldom-considered predicament in
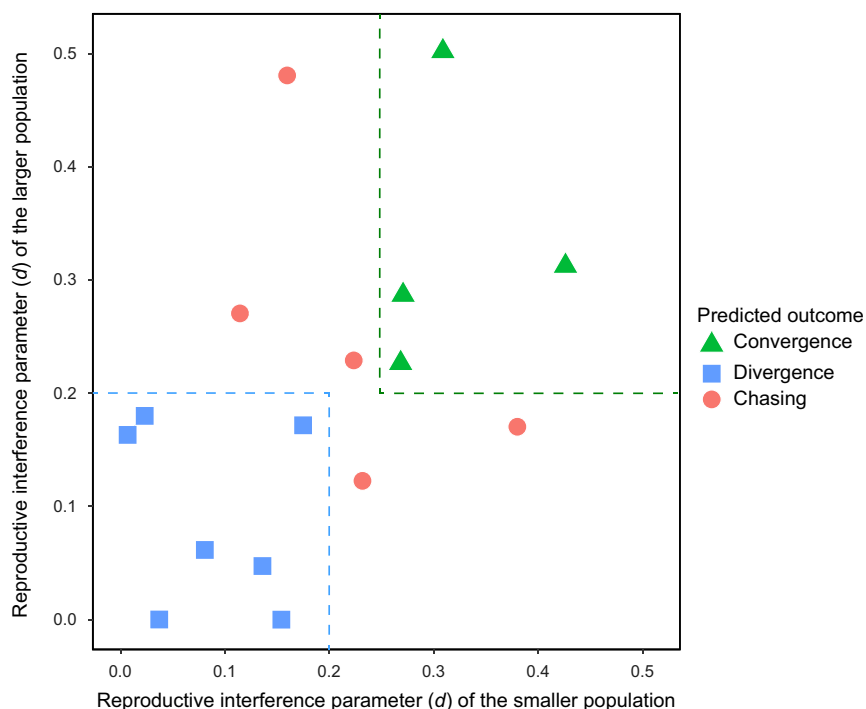

Figure 1 Predicted outcomes of secondary contact between *Hetaerina* species with the observed levels of reproductive interference. See Figs S2–S7 for examples of each type of outcome.
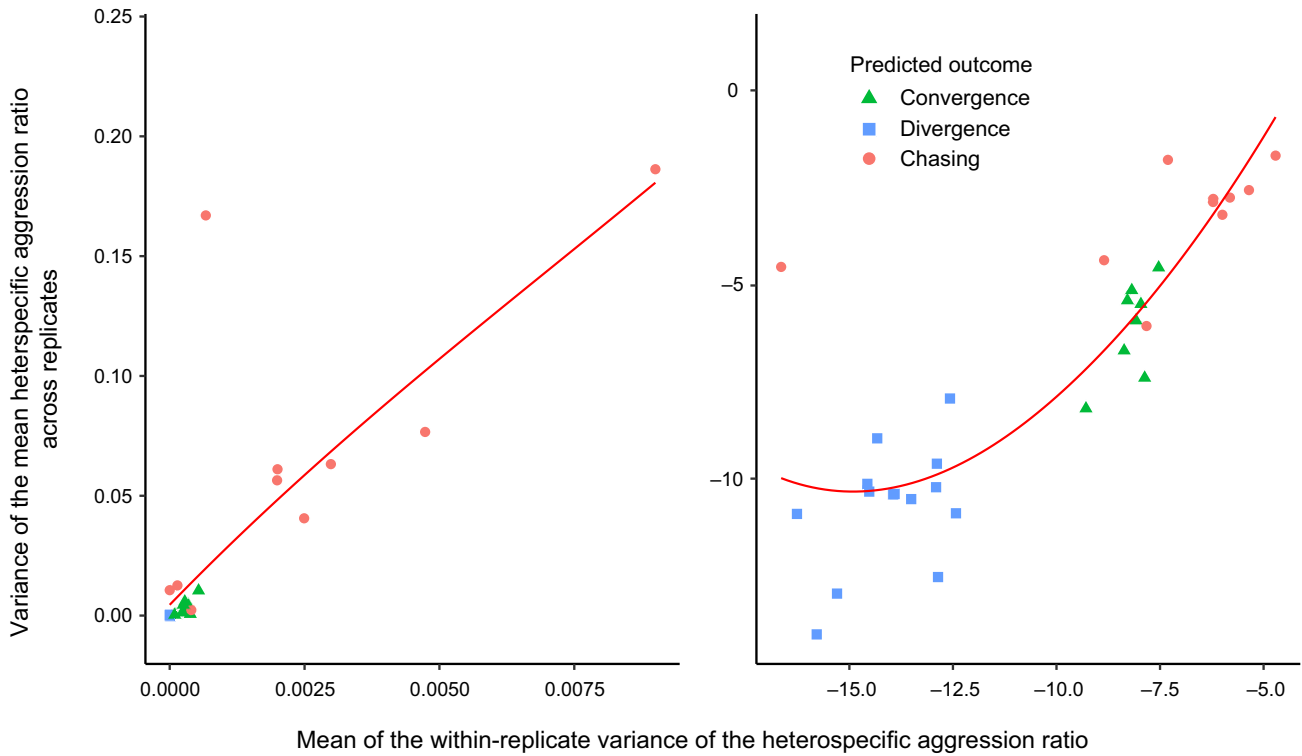
Figure 2 Relationship between variation in the mean heterospecific aggression ratio across replicates and temporal variation in the same ratio within replicates over the last 1ky of the ACD model simulations. In general, parameter combinations that led to different outcomes in different replicates also showed high variability within replicates. The upper left point is an exception: one population showed low variability within replicates but high variability across replicates. All cases with high variability across replicates are those in which chasing occurred due to an asymmetry in reproductive interference that crosses the divergence-convergence boundary (see Fig. 1). On a log scale (right panel), it is evident that the cases in which divergence occurred showed the lowest variability, which makes sense because once the species are outside each other's recognition range, small shifts in the trait values do not affect heterospecific aggression. The convergence cases showed much lower variability than the majority of cases in which chasing occurred, but it is understandable that convergent populations are more variable than divergent populations because when the species have similar trait values, small shifts in the trait values can have large effects on heterospecific recognition.
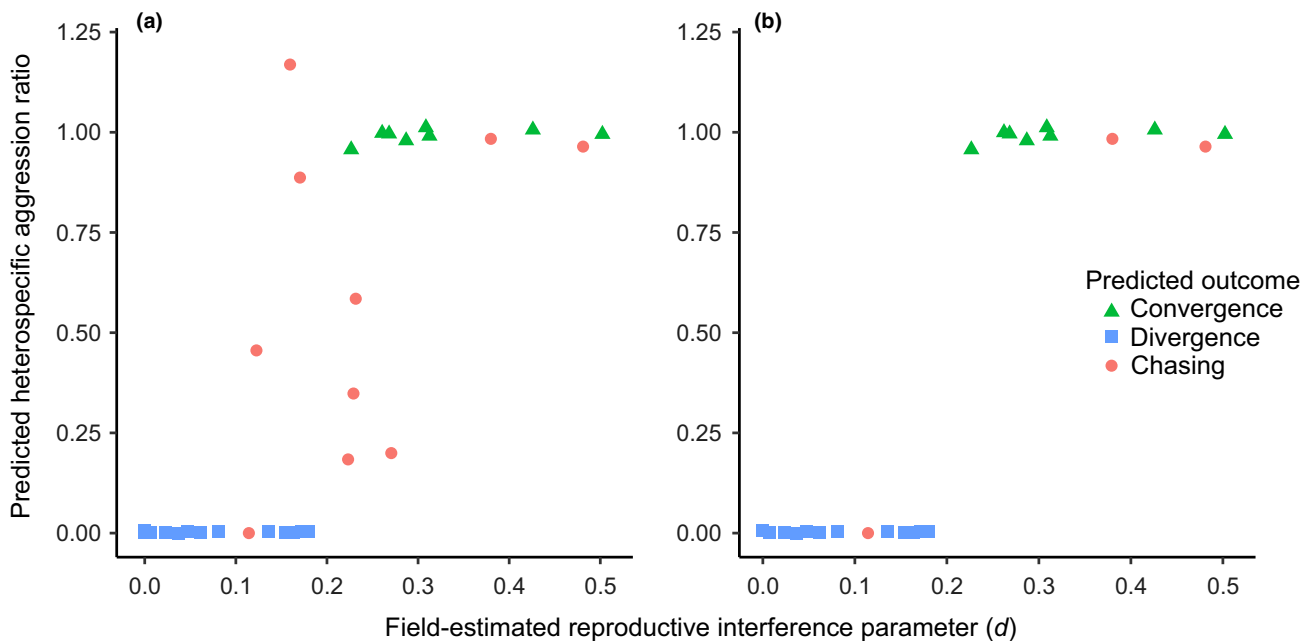
Figure 3 Relationship between field estimates of reproductive interference (*d*) and the ratio of heterospecific to conspecific aggression predicted by the ACD model. Predictions for all 32 populations are shown in a, but some of the predicted values are unstable. In b, seven cases with unstable predicted values are excluded.
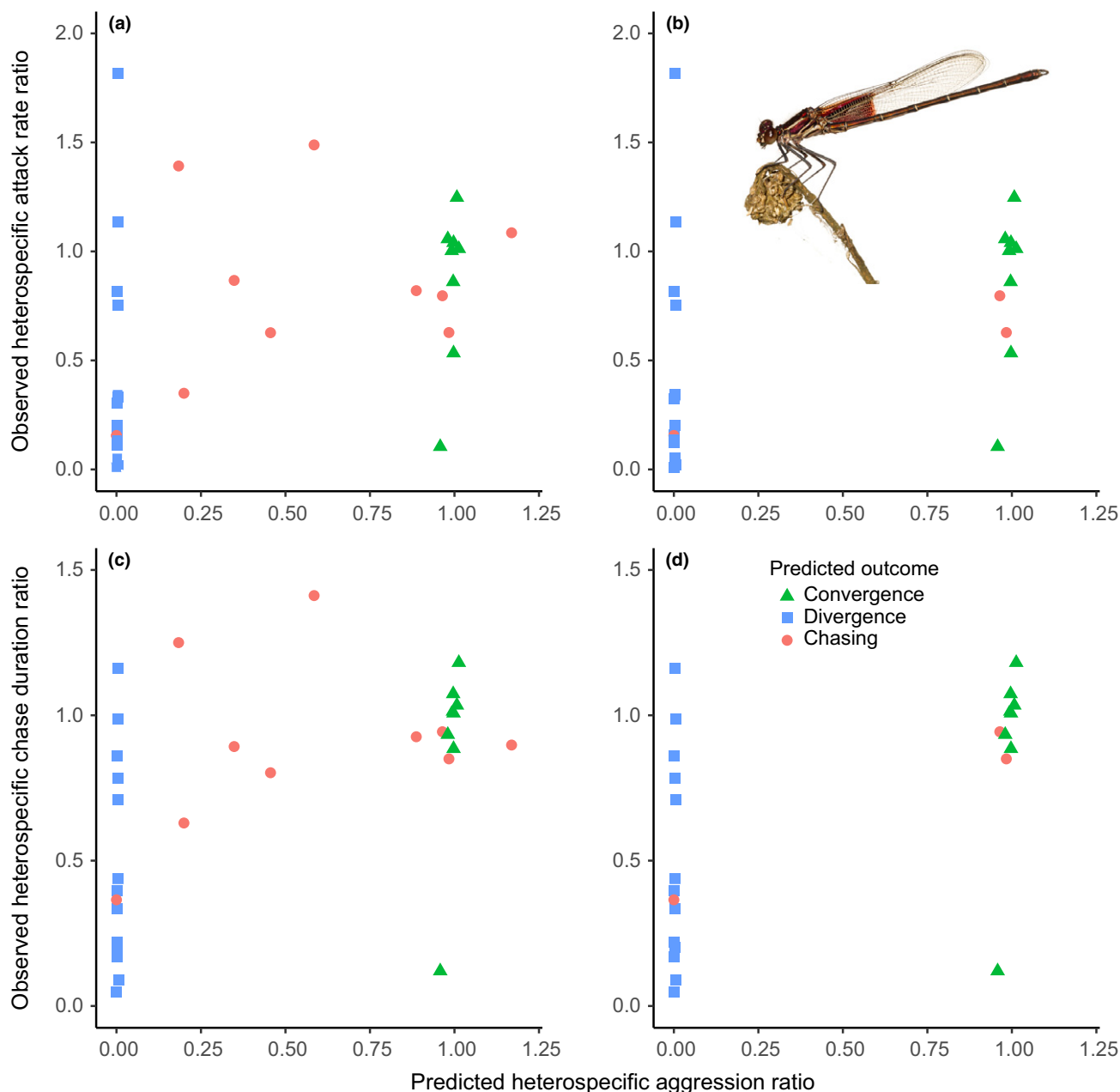
Figure 4 Relationship between observed and predicted heterospecific aggression ratios. In panels a and b, the observed heterospecific aggression ratio is based on the rates of physical attacks. In panels c and d, the observed heterospecific aggression ratio is based on the proportion of time territory holders chased intruders. Panels a and c include all populations; panels b and d exclude populations with unstable predictions. Inset photo of male *Hetaerina americana* by Neil Losin.

evolutionary biology (Blount *et al*. 2018). Nevertheless, 80% of the model's stable predictions matched levels of heterospecific aggression (HA) observed in the field. In addition to bolstering the evidence that ACD is a predictable phenomenon, this paper introduces a new, predictive approach to testing character displacement theory that, if applied to other systems, could help resolve long-standing questions about the importance of character displacement processes in nature (Pfennig & Pfennig 2012; Stuart & Losos 2013; Germain *et al*. 2018).

The traditional process-of-elimination approach to studying character displacement remains the best way to

determine whether an observed geographic pattern is likely to have been caused by a character displacement process (Schluter 2000), but eliminating all alternative explanations can be daunting (Dayan & Simberloff 2005). Another well-known problem is that character displacement processes need not leave an extant pattern of trait variation (Grant 1972; Lemmon *et al*. 2004; Goldberg & Lande 2006; Germain *et al*. 2018). Hence, the absence of a geographic pattern cannot be taken as evidence that character displacement has not occurred. Germain *et al*. (2018) proposed an alternative approach to studying ECD that involves measuring the strength of resource competition between

Table 1 Summary of predicted and observed outcomes. Heterospecific aggression ratios < 0.5 are categorised as 'low' and those ≥ 0.5 are categorised as 'high'. Unstable predictions are displayed in parentheses. See Table S1 for species names and site locations, and Table S5 for the numerical values used to generate this table

Site code	Predicted heterospecific aggression ratio of sp1	Predicted heterospecific aggression ratio of sp2	Observed heterospecific aggression ratio of sp1	Observed heterospecific aggression ratio of sp2	Species for which predicted and observed outcomes match
BC	High	High	High	High	Both
CT	High	High	High	High	Both
CV	Low	Low	Low	Low	Both
ES	(Low)	(low)	High	High	Neither
GO1	Low	Low	High	Low	sp2
GO2	Low	Low	Low	Low	Both
GO3	Low	Low	Low	High	sp1
LH	(High)	(Low)	High	High	sp1
OT	Low	Low	Low	Low	Both
PA	Low	Low	Low	Low	Both
PA1	Low	(low)	Low	Low	Both
PA2	Low	Low	Low	Low	Both
PX	High	High	High	High	Both
RB	High	High	High	Low	sp1
RS	(High)	High	High	High	Both
RT	High	(High)	High	High	Both
SL	Low	Low	High	High	Neither

populations in sympatry vs. allopatry and does not require measuring shifts in specific traits. Their approach is likely to reveal cases of character displacement that would otherwise be missed, and could provide information on the prevalence of character displacement in a clade. However, the occurrence of character displacement is still inferred from an observed pattern, as opposed to character displacement theory being used to predict where particular patterns should be found.

The predictive approach to studying character displacement exemplified by the current study fills an important gap between the scale of traditional studies of individual species pairs and large phylogenetic comparative studies. In large comparative studies, species pairs that deviate from the overall trends might illustrate genuine, alternative evolutionary outcomes, but instead tend to be regarded merely as outliers. For example, a phylogenetically robust pattern of song convergence in ovenbirds (Furnariidae) has been attributed to convergent ACD caused by resource competition (Tobias *et al.* 2014), but within this large clade (> 300 species), there might also be cases of song divergence caused by RCD or divergent ACD. Without a basis for predicting convergence in some species pairs and divergence in others, emphasising the overall trends is logical and necessary – it would be impractical to measure resource competition and reproductive interference in every species pair. We have shown that this is practical, however, at an intermediate taxonomic scale, with character displacement theory making different predictions for different species pairs.

In the four cases where our model's predictions clearly were not upheld, HA was higher than predicted. A plausible explanation for this apparent directional bias is that high HA is the ancestral state for sympatric *Hetaerina* populations and low HA, when observed, is a derived state. This explanation is consistent with previously documented patterns of divergence in male wing coloration and competitor

recognition in sympatry compared to allopatry in species pairs with low reproductive interference (Anderson & Grether 2010a,b). Why some sympatric populations with low reproductive divergence have not diverged from each other remains to be determined. Possible reasons include evolutionary time lag (if secondary contact occurred recently) and gene flow from allopatry swamping selection in sympatry, although most *Hetaerina* populations are strongly differentiated genetically (see Appendix of Drury *et al.* 2019).

Our results also highlight the importance of explicit demography in predictive studies of character displacement. Two factors affect net levels of reproductive interference in our model: the cost of sharing space with heterospecific males (d) and relative population density. The population with lower density experiences more frequent interspecific encounters per capita and thus is under stronger selection to converge or diverge, depending on whether d is on the convergence or divergence side of the threshold. Conversely, smaller populations may respond less to selection because beneficial mutations arise less frequently and are more readily lost to genetic drift and demographic stochasticity. Evidence for these opposing effects of population size was detected in our simulations (Figs S2–S7), but how these factors play out to affect the evolutionary dynamics in nature merits further study.

While we confirm that interspecific aggression in rubyspot damselflies can largely be explained as an evolved response to reproductive interference, why does reproductive interference itself persist? For that matter, why is reproductive interference so common in animals generally (Gröning & Hochkirch 2008; Grether *et al.* 2017; Shuker & Burdfield-Steel 2017)? An explanation that applies to many taxa in which males initiate mating is that the costs of missed mating opportunities are higher, for males, than the costs of attempting to mate with heterospecific females (Parker &

Partridge 1998; Ord *et al.* 2011; Takakura *et al.* 2015). If the females of different species are too similar phenotypically for males to profitably distinguish between them during secondary contact, this can result in an evolutionary dilemma or 'catch-22' in which reproductive interference persists because male mate recognition cannot evolve until female phenotypes diverge further, and vice versa (Drury *et al.* 2019). The evidence for this in rubyspot damselflies is that species differences in female coloration are strongly predictive of reproductive interference (Drury *et al.* 2015) and sympatric populations are no more reproductively isolated, behaviourally, than allopatric populations (Drury *et al.* 2019).

Our results for rubyspot damselflies are significant because they show that agonistic character displacement theory can be used to make non-trivial, population-specific predictions about the evolution of interspecific aggression based only on field estimates of reproductive interference and population densities. We anticipate that applying the same approach to other tractable organisms, including species that compete for common resources (e.g., food, nesting sites), will help shift the study of character displacement from its traditional focus on whether character displacement has occurred in specific cases to a more predictive science.

AUTHORSHIP

GFG and KWO developed the theoretical model, KWO wrote the code and GFG ran the simulations. GFG, JPD and CNA designed the field experiments. CNA, GFG, JPD and SM collected field data, supervised field assistants and transcribed field data. GFG and JPD devised the Monte Carlo simulation and JPD and KWO wrote and ran the code. GFG analysed the data and wrote the manuscript; all authors helped in revising the manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.rm8k9q0.

REFERENCES

Anderson, C.N. & Grether, G.F. (2010a). Character displacement in the fighting colours of *Hetaerina* damselflies. *Proc. R. Soc. Biol. Sci. Ser. B*, 277, 3669–3675.

Anderson, C.N. & Grether, G.F. (2010b). Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies. *Proc. R. Soc. Biol. Sci. Ser. B*, 277, 549–555.

Baker, M.C. (1991). Response of male indigo and lazuli buntings and their hybrids to song playback in allopatric and sympatric populations. *Behaviour*, 119, 225–242.

Blount, Z.D., Lenski, R.E. & Losos, J.B. (2018). Contingency and determinism in evolution: replaying life's tape. *Science (80-.)*, 362, eaam5979.

Brown, W.L. Jr & Wilson, E.O. (1956). Character displacement. *Syst. Zool.*, 5, 49–64.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, 18, 119–125.

Case, T.J. & Gilpin, M.E. (1974). Interference competition and niche theory. *Proc. Natl Acad. Sci. USA*, 71, 3073–3077.

Cody, M.L. (1969). Convergent characteristics in sympatric species - a possible relation to interspecific competition and aggression. *Condor*, 71, 223–239.

Dayan, T. & Simberloff, D. (2005). Ecological and community-wide character displacement: the next generation. *Ecol. Lett.*, 8, 875–894.

DeAngelis, & D.L., Mooij, W. (2005). Individual-based modeling of ecological and evolutionary processes. *Annu. Rev. Ecol. Evol. Syst.*, 36, 147–168.

Diamond, J.M. (1982). Mimicry of friarbirds by orioles. *Auk*, 99, 187–196.

Drury, J.P. & Grether, G.F. (2014). Interspecific aggression, not interspecific mating, drives character displacement in the wing coloration of male rubyspot damselflies (*Hetaerina*). *Proc. Biol. Sci.*, 281, 20141737.

Drury, J.P., Okamoto, K.W., Anderson, C.N. & Grether, G.F. (2015). Reproductive interference explains persistence of aggression between species. *Proc. R. Soc. B*, 282, 20142256.

Drury, J., Anderson, C., Castillo, M.C., Fisher, J., McEachin, S. & Grether, G.F. (2019). A general explanation for the persistence of reproductive interference. *Am. Nat.*, 194, 268–275.

Garrison, R.W. (1990). A synopsis of the genus *Hetaerina* with descriptions of four new species (Odonata: Calopterygidae). *Trans. Am. Entomol. Soc.*, 116, 175–259.

Germain, R.M., Williams, J.L., Schluter, D. & Angert, A.L. (2018). Moving character displacement beyond characters using contemporary coexistence theory. *Trends Ecol. Evol.*, 33, 74–84.

Gill, D.E. (1974). Intrinsic rate of increase, saturation density, and competitive ability. 2. Evolution of competitive ability. *Am. Nat.*, 108, 103–116.

Goldberg, E.E. & Lande, R. (2006). Ecological and reproductive character displacement on an environmental gradient. *Evolution*, 60, 1344–1357.

Grant, P.R. (1972). Convergent and divergent character displacement. *Biol. J. Linn. Soc.*, 4, 39–68.

Grether, G.F. (1996). Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution*, 50, 1949–1957.

Grether, G.F., Losin, N., Anderson, C.N. & Okamoto, K. (2009). The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol. Rev.*, 84, 617–635.

Grether, G.F., Anderson, C.N., Drury, J.P., Kirschel, A.N.G., Losin, N., Okamoto, K. *et al.* (2013). The evolutionary consequences of interspecific aggression. *Ann. N. Y. Acad. Sci.*, 1289, 48–68.

Grether, G.F., Peiman, K.S., Tobias, J.A. & Robinson, B.W. (2017). Causes and consequences of behavioral interference between species. *Trends Ecol. Evol.*, 32, 760–772.

Gröning, J. & Hochkirch, A. (2008). Reproductive interference between animal species. *Q. Rev. Biol.*, 83, 257–282.

Hairston, N.G. (1983). Alpha-selection in competing salamanders - experimental-verification of an a priori hypothesis. *Am. Nat.*, 122, 105–113.

Kirschel, A.N.G., Seddon, N. & Tobias, J.A. (2019). Range-wide spatial mapping reveals convergent character displacement of bird song. *Proc. R. Soc. B Biol. Sci.*, 286, 20190443.

Lemmon, A.R., Smadja, C. & Kirkpatrick, M. (2004). Reproductive character displacement is not the only possible outcome of reinforcement. *J. Evol. Biol.*, 17, 177–183.

Lorenz, K. (1962). The function of colour in coral reef fishes. *Proc. R. Inst. Gt. Britain*, 39, 282–296.

Moran, R.L. & Fuller, R.C. (2018a). Agonistic character displacement of genetically based male colour patterns across darters. *Proc. R. Soc. B Biol. Sci.*, 285, 20181248.

Moran, R.L. & Fuller, R.C. (2018b). Male-driven reproductive and agonistic character displacement in darters and its implications for speciation in allopatry. *Curr. Zool.*, 64, 101–113.

Nishikawa, K.C. (1987). Interspecific aggressive behaviour in salamanders: species-specific interference or misidentification? *Anim. Behav.*, 35, 263–270.

Okamoto, K.W. & Grether, G.F. (2013). The evolution of species recognition in competitive and mating contexts: the relative efficacy of alternative mechanisms of character displacement. *Ecol. Lett.*, 16, 670–678.

Ord, T.J., King, L. & Young, A.R. (2011). Contrasting theory with the empirical data of species recognition. *Evolution*, 65, 2572–2591.

Parker, G.A. & Partridge, L. (1998). Sexual conflict and speciation. *Philos. Trans. R. Soc. B. Biol. Sci.*, 353, 261–274.

Pasch, B., Sanford, R. & Phelps, S.M. (2017). Agonistic character displacement in social cognition of advertisement signals. *Anim. Cogn.*, 20, 267–273.

Payne, R.B. (1980). Behavior and songs of hybrid parasitic finches. *Auk*, 97, 118–134.

Peiman, K.S. & Robinson, B.W. (2007). Heterospecific aggression and adaptive divergence in brook stickleback (*Culaea inconstans*). *Evolution*, 61, 1327–1338.

Pfennig, D.W. & Pfennig, K.S. (2012). *Evolution's Wedge. Competition and the Origins of Diversity*. University of California Press, Berkeley, CA.

Reichert, M.S. & Gerhardt, H.C. (2014). Behavioral strategies and signaling in interspecific aggressive interactions in gray tree frogs. *Behav. Ecol.*, 25, 520–530.

Reif, J., Jiran, M., Reifovác, R., Vokurková, J., Dolatad, P.T., Petrusek, A. *et al.* (2015). Interspecific territoriality in two songbird species: potential role of song convergence in male aggressive interactions. *Anim. Behav.*, 104, 131–136.

Schluter, D. (2000). Ecological character displacement in adaptive radiation. *Am. Nat.*, 156, S4–S16.

Sedlacek, O., Cikanova, B. & Fuchs, R. (2006). Heterospecific rival recognition in the Black Redstart (*Phoenicurus ochruros*). *Ornis Fenn.*, 83, 153–161.

Shuker, D.M. & Burdfield-Steel, E.R. (2017). Reproductive interference in insects. *Ecol. Entomol.*, 42, 65–75.

Souriau, A., Kohoutová, H., Reif, J., Vokurková, J., Petrusek, A., Reifová, R. *et al.* (2018). Can mixed singing facilitate coexistence of closely related nightingale species? *Behav. Ecol.*, 29, 925–932.

Stuart, Y.E. & Losos, J.B. (2013). Ecological character displacement: glass half full or half empty? *Trends Ecol. Evol.*, 28, 402–408.

Takakura, K.-I., Nishida, T. & Iwao, K. (2015). Conflicting intersexual mate choices maintain interspecific sexual interactions. *Popul. Ecol.*, 57, 261–271.

Tobias, J.A. & Seddon, N. (2009). Signal design and perception in *Hypocnemis* antbirds: evidence for convergent evolution via social selection. *Evolution*, 63, 3168–3189.

Tobias, J.A., Cornwallis, C.K., Derryberry, E.P., Claramunt, S., Brumfield, R.T. & Seddon, N. (2014). Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. *Nature*, 506, 359–363.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.