

EXPERIMENTAL EVIDENCE FOR THE EVOLUTION OF INDIRECT GENETIC EFFECTS: CHANGES IN THE INTERACTION EFFECT COEFFICIENT, Ψ (Ψ), DUE TO SEXUAL SELECTION

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Indirect genetics effects (IGEs)—when the genotype of one individual affects the phenotypic expression of a trait in another—may alter evolutionary trajectories beyond that predicted by standard quantitative genetic theory as a consequence of genotypic evolution of the social environment. For IGEs to occur, the trait of interest must respond to one or more indicator traits in interacting conspecifics. In quantitative genetic models of IGEs, these responses (reaction norms) are termed interaction effect coefficients and are represented by the parameter ψ (ψ). The extent to which ψ exhibits genetic variation within a population, and may therefore itself evolve, is unknown. Using an experimental evolution approach, we provide evidence for a genetic basis to the phenotypic response caused by IGEs on sexual display traits in *Drosophila serrata*. We show that evolution of the response is affected by sexual but not natural selection when flies adapt to a novel environment. Our results indicate a further mechanism by which IGEs can alter evolutionary trajectories—the evolution of interaction effects themselves.

KEY WORDS: Cuticular hydrocarbons, *Drosophila serrata*, experimental evolution, interaction effect coefficient, natural selection, sexual selection.

Indirect genetic effects (IGEs) occur when the phenotype of an individual is, in part, determined by the genotype of a conspecific individual with which it interacts (Moore et al. 1997; Wolf et al. 1998). IGEs extend the classic view of phenotypes as being due to a combination of direct genetic and environmental influences (Falconer and Mackay 1996; Lynch and Walsh 1998) by recognizing that during social interactions, one individual provides the

environment for the other, meaning that the environment has a genetic component. By including a link between genotypes in the surrounding environment and focal phenotypes, IGEs broaden the pathway by which genes can influence phenotypic variation. Central to the theoretical study of IGEs has been their potential to alter the evolutionary dynamics of the traits involved. For example, because the environment contains genes, it can evolve, thereby creating an additional evolutionary process influencing IGE-affected traits (Wolf et al. 1998).

For IGEs to occur, the particular trait(s) of the interacting individual that act as the environmental “cue” or “effector” must

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have a genetic basis. The direction and extent to which the focal trait, z_1 , changes as a consequence of exposure to the cue trait, z_2 , is quantified by an “interaction effect coefficient” ψ (ψ), a key parameter in quantitative genetic models of IGEs (Moore et al. 1997). ψ has parallels with m in models of maternal effects (Kirkpatrick and Lande 1989), a specific example of an IGE, and more generally, reaction norms that are central to the study of phenotypic plasticity (Scheiner 1993). In essence, ψ measures the effect of the cue trait z_2 on the focal trait z_1 . If ψ is zero, the focal trait is unaffected by IGEs and the response to selection of z_1 can be modeled using the standard breeder’s equation (Lande 1979). To date, ψ has been considered as a fixed population parameter in quantitative genetic models of IGEs (Moore et al. 1997). However, it is likely that genetic variation may exist within a population for ψ , meaning that ψ itself can respond to selection. Evolutionary changes in ψ may therefore provide a second, and as yet unexplored, mechanism by which IGEs can alter evolutionary trajectories (Moore et al. 1997). Outside the realm of maternal effects, empirical study of ψ has been rare, but in two cases in which it has been estimated, ψ differed between genotypes (Kent et al. 2008; Bleakley and Brodie 2009). These recent studies suggest that ψ itself has evolutionary potential, although direct empirical demonstration is lacking and the types of selection that may affect its evolution remain unexplored (Ritchie 2008). Here, we took an experimental evolution approach to test whether interaction effects can evolve and importantly, to assess what kinds of selective processes might lead to their evolution.

The cuticular lipids of *Drosophila* provide an ideal system to test these ideas. Cuticular hydrocarbons (CHCs) are important in mediating social interactions in *Drosophila* with demonstrated influences on both species and mate recognition (Jallon 1984; Blows and Allan 1998; Higgie et al. 2000; Etges and Tripodi 2008). Recent work has demonstrated that these highly plastic traits are affected by IGEs during courtship and other social interactions in at least two species: *D. melanogaster* (Kent et al. 2008; Krupp et al. 2008) and *D. serrata* (Petfield et al. 2005). In *D. serrata*, the CHC phenotypes of males have been shown to change rapidly (within minutes) when courting a female in the presence of another male. The resulting IGEs on male CHCs were associated with genetic variance in both CHCs and body condition in females, indicating that either or both traits may serve as the cue to which males respond (Petfield et al. 2005).

Here, we have used an experimental evolution approach in which replicate populations of *D. serrata* were allowed to evolve in a novel larval food environment known to lead to CHC evolution (Rundle et al. 2006). The experiment employed a factorial design that independently manipulated the opportunities for natural and sexual selection, resulting in three populations under each evolutionary treatment combination. After 16 generations, we performed a social interaction assay in which we monitored

CHC expression of adult males within two social contexts: solitary (held in isolation), and interacting with a female and another male (held, and potentially mated with, a female from a standard genetic background). Because males from all populations were presented with standard females, evolutionary changes in ψ are implicated if the phenotypic consequences of social interactions vary among treatments. By testing for an interaction between the effects due to evolutionary treatments and the social environment, we were able to determine whether the consequences of social interactions had evolved in the novel environment in a response to natural selection, sexual selection, or both.

Material and Methods

EVOLUTIONARY MANIPULATION

We investigated the response of ψ to natural and sexual selection using an experimental evolution approach. The full details of the creation of the experimental lines are outlined in detail elsewhere (Rundle et al. 2006). In brief, 12 replicate populations were derived from a stock population of *D. serrata* and were randomly assigned in a two-way factorial design to one of four experimental treatments. These treatments independently manipulated the opportunities for natural and sexual selection (Blows 2002), resulting in three populations that experienced both natural and sexual selection, three that experienced reduced natural selection with sexual selection present, three that experienced natural selection with reduced sexual selection, and three that experienced reduced natural and reduced sexual selection. All populations were cultured for 16 nonoverlapping generations on a novel corn-based food media (Rundle et al. 2005) by transferring 50–55 mated females each generation.

The selection treatments were implemented as follows. Natural selection was permitted by allowing individual females to contribute to the next generation in proportion to the number of adult progeny she produced (natural selection present) or greatly reduced by equalizing the contribution of all females. Sexual selection was permitted by mixing virgin adults for three to six days for mating every generation, or greatly reduced by enforcing random monogamy. The success of these treatments in manipulating natural and sexual selection has been confirmed by significant effects on the evolution of CHC sexual dimorphism (Chenoweth et al. 2008), productivity (Rundle et al. 2006), and female mate preferences (Rundle et al. 2009).

SOCIAL INTERACTION ASSAY

Direct empirical estimation of ψ is particularly demanding because it requires repeated exposure of genetically identical (Bleakley and Brodie 2009; Danielson-Francois et al. 2009), or at least related (McGlothlin and Brodie 2009), focal individuals to multiple conspecifics that vary genetically in their cue trait, such that the covariance between the cue and the focal trait can be estimated. In

D. serrata, the exact cue trait is unknown, although female CHC phenotype and body condition have been implicated (Petfield et al. 2005). Here, we have taken a population-level approach that does not rely on knowing the precise identity of the female cue. This approach uses a social interaction assay in which the average CHC phenotype of males within and among selection lines is compared for two social environments: solitary or interacting with females from a standard genetic background (the stock population). Significant evolution of ψ is inferred from an interaction between experimental treatment and social environment.

That the evolution of ψ can be inferred from the experimental treatment \times social environment interaction can be seen from the perspective of IGE theory, where the value of an IGE-affected trait z_1 is influenced by a “cue” trait z_2 expressed in a conspecific individual in the social environment (in our case the female). Assuming no reciprocal effects, the trait value for z_1 in a focal individual can be described by

$$z_1 = a_1 + e_1 + \psi_{12}(a'_2 + e'_2), \quad (1)$$

where a_1 and e_1 are the direct additive genetic and environmental effects, respectively on z_1 . The influence on z_1 due to the IGE is given by the interaction effect coefficient ψ_{12} , with a'_2 and e'_2 representing the additive and environmental effects, respectively on the “cue” trait, z_2 (i.e., $z_2 = a'_2 + e'_2$) (eq. 5a Moore et al. (1997)). In our design, we randomly sampled females from a common genetic background and conducted all tests within a common laboratory environment, meaning that neither a'_2 nor e'_2 are expected to differ systematically among selection treatments. Therefore, the only factor expected to give rise to an interaction between social and selection treatments is genetic variation in ψ_{12} among selection treatments.

The social interaction assay consisted of either holding randomly selected males from the experimental populations as solitary, or allowing them to interact socially with two other flies—another male from the same experimental population and a female from the stock population. Experimental males for use in the trials were collected as virgins using light CO₂ anesthesia from the offspring produced by parents that had experienced one generation of a common selective treatment (reduced natural and reduced sexual selection). Females were collected from offspring of stock parents raised in the novel corn-food environment. All virgin offspring were held individually in vials for four to seven days after collection before use in the assay. During the assay, individuals remained in both treatments until the female in the social treatment mated with one of the two males, at which point the focal male was removed for CHC extraction.

For the focal male, there are two outcomes of interacting in this arena: he will either mate successfully with the female or be rejected by her. Male CHCs are well known to affect male mating success in this species (Hine et al. 2002; Chenoweth and Blows

2003), so there was the potential for a confounding effect due to “female choice.” We therefore ensured that an equal number of chosen and rejected males entered the “social” treatment and, to avoid dependence between chosen and rejected males, we used only one male from each trial, discarding the other. In total, we performed 40 mating trials per line (resulting in CHC samples from 20 rejected males and 20 chosen males) and also processed 40 solitary virgin males from each line at the same time. The solitary virgin males were transferred to fresh vials at the same time as the social trials were set up. This was to remove any possible phenotypic differences between treatments due to a “new environment” rather than the social effect.

Because previous work had indicated that female CHCs could act as a “cue trait” initiating IGEs in male *D. serrata* (Petfield et al. 2005), we also wanted to determine whether these traits had responded to the experimental manipulation. When setting up the social interaction assay, we therefore sexed 20 virgin females from each line and held them singly in vials for subsequent CHC analysis.

CHC ANALYSIS

Nine CHCs were analyzed for each male employing standard gas chromatography methods: Z,Z-5,9-C_{24:2}, Z,Z-5,9-C_{25:2}, Z-9-C_{25:1}, Z-9-C_{26:1}, 2-Me-C₂₆, Z,Z-5,9-C_{27:2}, 2-Me-C₂₈, Z,Z-5,9-C_{29:2}, and 2-Me-C₃₀ (Blows and Allan 1998). The areas under each of the nine chromatograph peaks were integrated and, to allow direct comparisons between previous work, were transformed into eight log-contrast values for subsequent statistical analyses (Blows and Allan 1998) following Atchinson (1986) and using Z,Z-5,9-C_{24:2} as the common divisor. More detail on the statistical treatment of CHCs can be found in Blows and Allan (1998). We used the Mahalanobis distance technique described in Sall et al. (2005) and implemented in the multivariate package JMP IN version 5.1 (SAS Institute, Cary, NC) for multivariate outlier detection. We aimed to phenotype 40 males from each level of the social treatment (solitary vs. social) in each of the 12 replicate populations that had undergone experimental evolution, with equal numbers of chosen and rejected males included in the “social” level of the social treatment. After accounting for unsuccessful GC samples and multivariate outliers, a total of 887 males were included in the final analysis.

STATISTICAL ANALYSIS

The univariate mixed effects model to partition the effects of natural and sexual selection and social environment on male CHC phenotypes was

$$\begin{aligned} CHC = & N + S + Social + N \times S + N \times Social \\ & + S \times Social + N \times S \times Social + replicate(N \times S) \\ & + Social \times replicate(N \times S), \end{aligned} \quad (2)$$

Table 1. Differences in fixed effects among models fitted to each CHC trait for evaluation. All models contain the random effect terms: *replicate*($N \times S$) and *Social* \times *replicate*($N \times S$). Refer to text for keys to model terms.

Model	Fixed effects
1	$N+S+Social+N \times S+N \times Social+S \times Social + N \times S \times Social$
2	$N+S+Social+N \times S+N \times Social+S \times Social$
3	$N+S+Social+N \times S+N \times Social$
4	$N+S+Social+N \times S+S \times Social$
5	$N+S+Social+N \times S$

where N and S refer to the manipulation of natural and sexual selection, respectively and *Social* represents the levels of the social interaction assay treatments (solitary and interacting). All effects were considered as fixed apart from *replicate*($N \times S$) and *Social* \times *replicate*($N \times S$), which were random. The ideal analysis would have been a multivariate version of the above model that considered all eight log-contrast CHCs simultaneously. However, due to the large number of traits considered for a modest number of replicates lines, the MANOVA could not be applied so we performed univariate analyses on each trait separately.

For each trait, we fitted five linear mixed effects models that differed in their fixed effect structures but shared both random effects (Table 1). The models considered reflected our key interests here in social effects and specifically, the interactions between any social effects and particular experimental selection treatments. Prior to significance testing, we performed model selection using Akaike's information criterion (AIC). The use of information-theoretic criteria has the advantage of allowing comparisons between nonnested models (Burnham and Anderson 2002, p. 88). All models were fitted using maximum likelihood to facilitate the comparison of AIC scores across models with different fixed effects structures (Littell et al. 2006) and the best model for each trait was then refitted using restricted maximum likelihood (REML). We fitted all models using the R statistical programming environment (Team 2008) using the *lmer* function from the *lme4* package (Bates et al. 2008). Significance of fixed and random effects (tested for best models only) was performed using the Markov chain Monte Carlo (MCMC) approach implemented in the *languageR* package using the default setting of 10,000 iterations (Baayen 2008). The MCMC approach is favored over F -ratio approximations because the latter are often anticonservative in mixed effects models when sample sizes (here, the number of independent replicate populations) are small (Baayen et al. 2008).

For the analysis of virgin female CHCs, the following linear model was applied:

$$CHC = N + S + Social + N \times S + replicate(N \times S). \quad (3)$$

Significance testing was performed as per the male analysis.

Results

The CHC blend of *D. serrata* consists of three different types of compound: monenes, dienes, and methyl-branched alkanes. For both the monenes and dienes, the best model (according to AIC) was model 5 (Table S1), which does not include any interactions between the social environment and the evolutionary treatments (Table 1). Subsequent significance testing of model 5 for each of these compounds using MCMC failed to detect any significant main effect of social environment or the experimental evolution treatments (Table 2).

By contrast, significant effects were detected for all three of the methyl-branched alkanes (Table 2). For the first, 2-MeC₂₆, the interaction effect in males evolved in response to changes in the opportunity for sexual selection (sexual selection \times social environment interaction: $P_{MCMC} = 0.041$). The main effect of sexual selection was also significant for this trait ($P_{MCMC} = 0.002$). In populations in which the opportunity for sexual selection was removed, the effect of social environment was limited with social males exhibiting a nonsignificant reduction in CHC concentration compared to solitary males, whereas males in the sexual selection treatment increased 2-MeC₂₆ concentration during social interaction (Fig. 1A). Post hoc pairwise comparisons suggested that the response was due to solitary males in the sexual selection treatment signaling from a lower baseline level of 2-Me-C₂₆ than males from the nonsexually selected lines (Fig. 1A).

The same overall pattern in trait means was mirrored by the second methyl-branched alkane, 2-MeC₂₈, although the interaction effect was marginally nonsignificant (sexual selection \times social environment interaction: $P_{MCMC} = 0.069$, Fig. 1B). The main effect of sexual selection was significant for this trait ($P_{MCMC} = 0.049$). Finally, for 2-MeC₃₀, the longest methyl-branched alkane in the blend, there was a highly significant effect of social environment ($P_{MCMC} = 0.006$), indicating its involvement in IGEs, but no interaction with sexual selection treatment was detected (Fig. 1C).

In females, one trait responded to the evolutionary manipulation of natural and sexual selection (Table S2). Again, this was the shortest of the methyl-branched alkanes, 2-MeC₂₆, for which there was a significant main effect of natural ($P_{MCMC} = 0.028$) but not sexual selection ($P_{MCMC} = 0.413$). The interaction was not significant. There was no indication that any other female trait responded to the manipulation.

Discussion

Our aim in this experiment was to determine whether the interaction effects due to IGEs could respond to either natural or sexual

Table 2. MCMC posterior probability values (P_{MCMC}) from best model fits for eight cuticular hydrocarbons in male *D. serrata* following 16 generations of experimental evolution. A dash indicates that a term was not present in the best model. The 95% highest posterior density intervals are shown for the variance components of the two random effects, *replicate*($N \times S$) and *Social* \times *replicate*($N \times S$). Significant terms are indicated in bold.

Effect	Monoenes		Dienes			Methylalkanes		
	9-C ₂₅	9-C ₂₆	5,9C ₂₅	5,9C ₂₇	5,9C ₂₉	2MeC ₂₆	2MeC ₂₈	2MeC ₃₀
Natural (N)	0.838	0.592	0.476	0.260	0.915	0.389	0.172	0.907
Sexual (S)	0.686	0.992	0.917	0.223	0.707	0.002	0.852	0.336
Social	0.961	0.895	0.664	0.415	0.472	0.468	0.049	0.006
S \times N	0.329	0.843	0.484	0.481	0.774	0.441	0.355	0.679
Rep(S \times N)	0.111–0.586	0.288–0.714	0.000–0.541	0.370–0.729	0.305–0.695	0.000–0.404	0.000–0.539	0.073–0.607
Social \times S	–	–	–	–	–	0.041	0.069	–
Social \times N	–	–	–	–	–	–	–	–
Social \times N \times S	–	–	–	–	–	–	–	–
Social \times Rep(S \times N)	0.000–0.310	0.113–0.377	0.093–0.399	0.069–0.322	0.237–0.490	0.139–0.404	0.135–0.437	0.000–0.336

selection with a change in environment. This was achieved using the system of contact pheromones composed of CHCs expressed during mating in the fruit fly, *D. serrata*. We used a 16-generation evolution experiment that independently manipulated the opportunities for natural and sexual selection. By exposing evolved males from this evolutionary manipulation to females representing a common stock genetic background, we could determine whether the modulations in male phenotype that occur during the social interactions of courtship and mating evolved during experimental evolution. We detected a pattern consistent with a response to sexual selection alone of the interaction effect coefficient, Ψ , for two methyl-branched alkanes.

That methyl-branched alkanes were involved in the responses seen here is consistent with previous results indicating their role in both sexual signaling and IGEs. Sexual selection due to female mate choice is commonly detected on methyl-branched alkanes in *D. serrata*, using both laboratory-reared (Hine et al. 2002; Chenoweth and Blows 2005) and field-caught males (Hine et al. 2004; Petfield et al. 2005). The involvement of this class of compound in mate choice is also consistent across much of the species natural distribution (Rundle et al. 2008), although the specific direction of sexual selection differs among populations. Previous laboratory study in *D. serrata* has also implicated the methyl-branched alkanes in IGEs. For example, 2-MeC₂₆ had the highest indirect genetic variance of all eight traits considered here (Petfield et al. 2005), implying that phenotypic variance in 2-MeC₂₆ was the most sensitive hydrocarbon to genotypic differences among females in the social environment. Our results in this experiment suggest a critical role for methyl-branched alkanes in the evolution of the signaling response of males.

There are at least two possible mechanisms by which an interaction effect may have evolved in our experimental lines. First,

the changes in male CHCs that occur during social interaction (i.e., their signal or display during courtship and/or mating) may have evolved in response to evolution of the female cue traits that males assess. If the female cues evolve, the male response may track the changes in the genotypic composition of their social environment. In *D. serrata*, the female cue traits are yet to be identified, although female condition, multiple CHCs, or both have been identified as potential candidates. In particular, quantitative genetic analysis showed that genetic variance in female condition explained 19.8% of the indirect genetic variance in male CHCs in *D. serrata*, and that genetic variation in the male signaling response was associated with genetic variation in all eight of the hydrocarbons considered here (Petfield et al. 2005). Although no data were available for female condition in the current experiment, we did see a significant response to selection in one CHC in females: the methyl-branched alkane 2-MeC₂₆. Although the evolution in females of the same trait for which the interaction effect evolved in males suggests an association, females CHCs evolved in response to natural selection, whereas the male display evolved in response to sexual selection. It therefore appears unlikely that the evolution of female CHCs cues were responsible for the observed changes in male signaling.

A second mechanism by which the interaction effect may have evolved is in response to the evolution of the baseline (i.e., nonsignaling) expression of male CHCs. In the current case, the presence of sexual selection alone in the novel environment appears to have caused solitary males to produce lower concentrations of 2-MeC₂₆ (Fig. 1) as compared to interacting males, producing a change in the nonsignaling baseline level of this trait. The consequences of social interaction therefore differed for males from these two evolutionary treatments. Males from the sexual selection present treatment rapidly increased 2-MeC₂₆

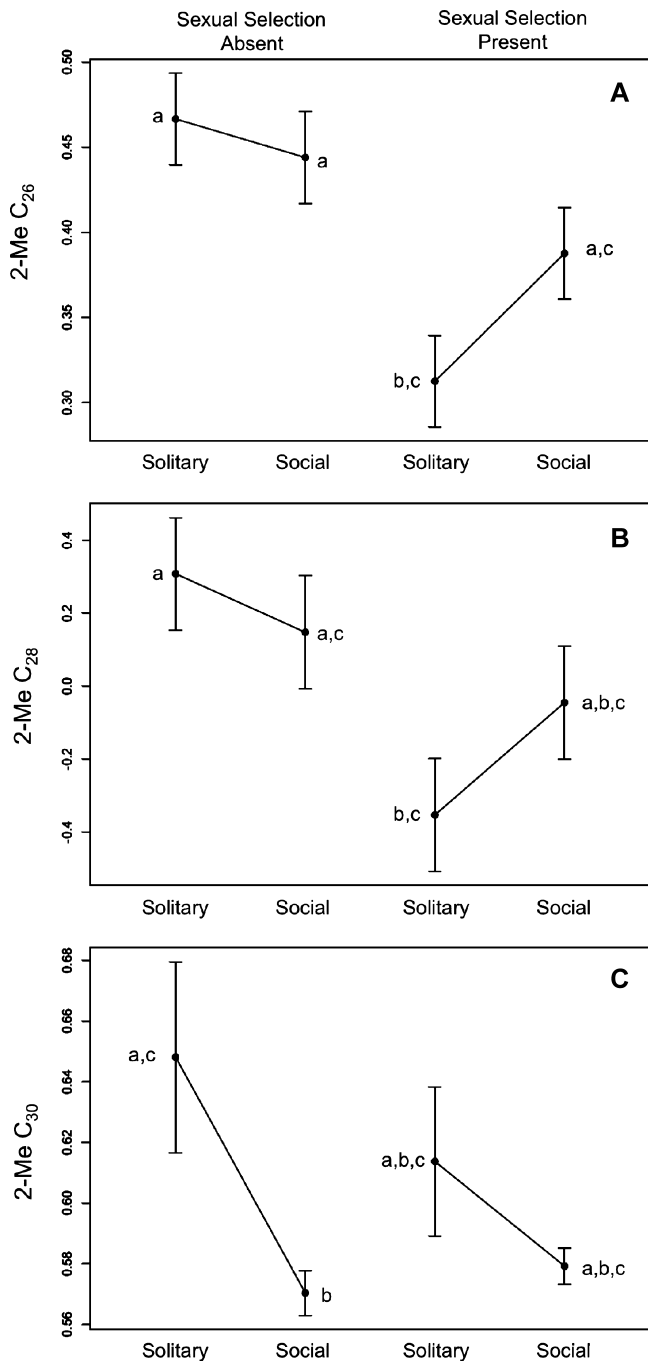


Figure 1. The effect of social environment on male *D. serrata* methyl-branched alkane expression (A: 2MeC₂₆, B: 2MeC₂₈ and C: 2MeC₃₀) among experimental lines that differed in opportunity for sexual selection following 16 generations of adaptation to a novel environment. Plotted are least squares means (± 1 SE). Means that share lowercase letters were not significantly different in pairwise post hoc comparisons after adjustment for multiple tests ($P > 0.05$).

expression when interacting socially, whereas males from the nonsexually selected lines did not alter 2-MeC₂₆ expression to any significant degree. Males from both treatments may have been signaling toward a common display phenotype, the achievement

of which required a change in 2-MeC₂₆ expression in the sexual selection treatment but not the nonsexually selected treatment. The apparent signaling by males of a common phenotype despite changes in baseline expression implies that this phenotype remains preferred during this social interaction (i.e., courtship and mating). An analysis of female mating preferences for male CHCs in these same experimental populations indicated that their evolution was minor and did not occur in response to sexual selection alone (Rundle et al. 2009), suggesting that males from these populations may have been trying to achieve a common phenotype preferred by females from both treatments.

A common warning when working on IGE-affected traits is that failing to consider the social context when measuring phenotypes can lead to misleading conclusions about the evolutionary dynamics of a trait (Chenoweth and Blows 2006; Wilson et al. 2009). This is particularly evident from the evolutionary response in 2-MeC₂₆: if one had assayed this trait in the interacting environment alone, one would likely have concluded that it had not responded to the evolutionary manipulation. However, this is clearly not the case when the alternative social context is considered. Our current approach to assessing the evolution of interaction effects during experimental evolution was at the scale of the overall population average effect. In the future it would be favorable to combine variance-component based quantitative genetic approaches with experimental evolution to track genetic variation in male traits and, by exposing males to a range of female genetic backgrounds, to estimate how genetic variance in ψ changes within and among populations experiencing different selection regimes.

Interaction effect coefficients in IGE models are analogous to reaction norms that describe plastic responses to changes in the physical environment but with the critical distinction that they describe change in phenotype in response to different levels of a social (and therefore heritable) environment. Although it has long been understood that reaction norms may evolve in both intercept and slope (Scheiner 1993, 2002), the analogous evolution of ψ has been predicted (Moore et al. 1997), but until now, not demonstrated. We have provided experimental evidence that IGEs can evolve via the interaction effect coefficient. Thus far, the theoretical study of IGEs has outpaced their empirical study (McGlothlin and Brodie 2009). The results reported here, demonstrating the evolution of interaction effects, suggest an additional mechanism by which indirect effects can shape phenotypic evolution that warrants further investigation.

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Supporting Information

The following supporting information is available for this article:

Table S1. AIC values for models 1–5 fitted to the eight cuticular hydrocarbons in male *D. serrata*.

Table S2. MCMC posterior probability values (P_{MCMC}) from analyses of female *D. serrata* CHCs following 16 generations of experimental evolution.

Supporting Information may be found in the online version of this article.

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