

Ecological divergence promotes the evolution of cryptic reproductive isolation

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Speciation can involve the evolution of ‘cryptic’ reproductive isolation that occurs after copulation but before hybrid offspring are produced. Because such cryptic barriers to gene exchange involve post-mating sexual interactions, analyses of their evolution have focused on sexual conflict or traditional sexual selection. Here, we show that ecological divergence between populations of herbivorous walking sticks is integral to the evolution of cryptic reproductive isolation. Low female fitness following between-population mating can reduce gene exchange between populations, thus acting as a form of cryptic isolation. Female walking sticks show reduced oviposition rate and lower lifetime fecundity following between-population versus within-population mating, but only for mating between populations using different host-plant species. Our results indicate that even inherently sexual forms of reproductive isolation can evolve as a by-product of ecological divergence and that post-mating sexual interactions do not necessarily evolve independently of the ecological environment.

Keywords: speciation; mating success; natural selection; sexual selection; walking sticks

1. INTRODUCTION

Speciation involves the evolution of barriers to gene exchange (reproductive isolation) between diverging populations. Understanding speciation thus involves two major tasks: determining which reproductive barriers were involved in the initial reduction in gene flow between populations, and understanding which evolutionary forces produced them (Coyne & Orr 2004).

Until recently, forms of reproductive isolation that act after copulation but before hybrids are produced have been less-explored than other types of barriers. Examples include poor transfer or storage of sperm (Price *et al.* 2001), failure of fertilization when gametes contact each other (Palumbi 1998; Swanson & Vacquier 2002), and reduced oviposition rates because foreign ejaculate or courtship behaviour fails to stimulate oviposition (Fuyama 1983; Gregory & Howard 1993; Price *et al.* 2001). These processes can lead to reduced reproductive output for between-population versus within-population matings, thereby decreasing gene exchange between populations. Such reproductive barriers are sometimes referred to as ‘cryptic reproductive isolation’, because they cannot be detected from mating probabilities or by examining hybrid fitness (Price *et al.* 2001; Coyne & Orr 2004). Despite recent examples of cryptic reproductive isolation, the processes that drive their evolution remain obscure (Coyne & Orr 2004). Selection has been implicated, but it remains unclear what forms of selection are involved.

Three main processes have been hypothesized to drive the evolution of cryptic reproductive isolation: sexual selection, reinforcing selection and natural selection

(Coyne & Orr 2004). Sexual selection may be particularly likely to cause cryptic reproductive isolation, because such isolation involves sexual interactions. Most work on speciation via sexual selection has focused on divergence in mate preferences, but the same predictions generally apply to cryptic isolation as well (Parker & Partridge 1998). In particular, sexual conflict between males and females has received much attention as a mechanism driving the evolution of forms of reproductive isolation that involve sexual interactions (Rice 1998; Parker & Partridge 1998; Gavrillets 2000; Martin & Hosken 2003; Knowles *et al.* 2004). However, the role of sexual conflict is controversial, because it may or may not promote reproductive isolation (Parker & Partridge 1998; Rowe *et al.* 2003). Furthermore, traditional sexual selection can generate similar predictions and can also lead to the evolution of reproductive isolation (Lande 1981; Panhuis *et al.* 2001; Pizzari & Snook 2003; Arnqvist 2004). However, because both sexual conflict and many forms of sexual selection are expected to operate independently from the ecological environment, divergence driven by either process leads to the expectation that reproductive isolation should occur for crosses between both ecologically similar and between ecologically divergent populations.

Second, a reinforcement-like process may cause cryptic reproductive isolation via selection against maladaptive hybridization (Coyne & Orr 2004). Under this scenario, greater divergence is expected between populations in geographical contact versus geographically separated populations, because maladaptive hybridization occurs only in the former situation (Servedio & Noor 2003).

Third, speciation may occur via divergent natural selection when reproductive isolation evolves as a pleiotropic by-product of populations adapting to different ecological environments (Schluter 2000; Funk *et al.* 2002;

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Table 1. Results of paired *t*-tests examining whether female fitness is reduced in between-population versus within-population crosses (see table 2 for means from individual pairs of populations). (Three different scenarios are examined: allopatric pairs of populations using the same host, parapatric pairs of populations using different hosts and allopatric pairs of populations using different hosts. Mean difference is calculated as within-population minus between-population. Analyses confined to only females originating from *Ceanothus* yielded trends in the same direction in all cases (significance levels for these analyses are shown in the extreme right-hand column).)

comparison	mean difference	test statistic	d.f.	<i>p</i> -value	<i>p</i> -value (C only)
<i>fecundity</i>					
allopatric same host	2.33	0.47	2	0.69	0.90
parapatric different host	4.25	0.72	7	0.49	0.38
allopatric different host	16.00	6.36	2	0.02	0.01
<i>oviposition rate</i>					
allopatric same host	0.00	0.00	2	1.00	0.88
parapatric different host	0.41	2.54	7	0.04	0.02
allopatric different host	0.60	0.60	2	0.03	0.04

Rundle & Nosil 2005). The central prediction of this 'ecological speciation' hypothesis is that ecologically divergent pairs of populations will exhibit greater reproductive isolation than ecologically similar pairs. If natural selection drives the evolution of cryptic reproductive isolation, then reproductive success should be reduced following between-population mating, but only for pairs of populations that are ecologically divergent. Research on ecological speciation has focused on habitat and mate preference, or the ecological fit of hybrids to the niches of parental species (Schluter 2000; Funk *et al.* 2002; Rundle & Nosil 2005). Cryptic reproductive isolation has received almost no attention in studies of ecological speciation, perhaps because it is assumed that this inherently sexual form of isolation evolves via non-ecological sexual selection or sexual conflict (but see Knowles *et al.* 2004). We note that ecological speciation can involve sexual selection, but only those forms that depend on the ecological environment (Endler 1992; Schluter 2000; Boughman 2002; see §4).

One approach to addressing which processes drive the evolution of reproductive isolation is to quantify reproductive isolation in crosses between populations under different ecological and geographical scenarios (Schluter 2000; Funk *et al.* 2002). However, this approach has yet to be systematically applied to the analysis of cryptic reproductive isolation. Here, we conducted crosses within and between pairs of *Timema* walking stick populations to test for the presence and causes of forms of cryptic reproductive isolation. Because these populations differ in both ecology (host-plant use) and geography (allopatry versus parapatry), their study allows partitioning of the effects of ecology and geography on reproductive isolation. The results show that ecological divergence between populations is integral to the evolution of cryptic reproductive isolation.

Timema are wingless, phytophagous insects inhabiting the chaparral of southwestern North America (Crespi & Sandoval 2000). Here, we focus on *Timema cristinae*, a species which feeds on two different host-plant species (*Adenostoma fasciculatum* and *Ceanothus spinosus*). The current study examines divergence between populations, where a 'population' is defined as all of the walking sticks collected within a homogenous patch of a single host-plant species (as in Nosil *et al.* 2002, 2003). Pairs of populations on different hosts are considered ecologically divergent,

whereas those on the same host are considered ecologically similar. Additionally, populations can be parapatric (i.e. in geographical contact with a population of insects adapted to the alternative host) or allopatric (geographically separated from all populations adapted to the alternative host).

We calculated total lifetime fecundity (number of eggs laid), longevity and oviposition rate of females used in between-population versus within-population crosses ($n = 31\ 369$ eggs from 689 crosses; table 2). A cross is a mating between a male and a female, thus when referring to female fitness we refer to the females used in the actual crosses (not their F_1 offspring). Reduced female fecundity, longevity or oviposition rate in between-population versus within-population crosses represents a partial barrier to gene exchange, because reproductive output is lower for between-population matings. Lifetime fecundity and longevity are standard measures of fitness. In *T. cristinae*, oviposition rate following mating likely also represents an important component of fitness. Females that mate with males from the alternative host likely suffer high rates of visual predation owing to less-cryptic males riding on their back for several days following copulation (Sandoval 1994a,b; Nosil *et al.* 2002, 2003; Nosil 2004). Thus, females engaging in between-host mating may live only a short time in nature such that a low rate of oviposition (i.e. fewer eggs per unit time) translates into reduced lifetime fitness. We note that even locally cryptic individuals are preyed upon (albeit at a lower rate than less-cryptic individuals) such that there are potential fitness costs to low oviposition rate even for within-host matings.

We can use the geographical and ecological variation among *T. cristinae* populations to evaluate three alternative predictions concerning the processes driving the evolution of their reproductive barriers. If sexual conflict or sexual selection acting independently from the ecological environment drives evolution, then female fitness should be reduced in between-population crosses even for pairs of populations that are not ecologically divergent (i.e. those on the same host). If reinforcing selection is important, then female fitness should be most reduced for crosses involving parapatric pairs of populations on different hosts. If divergent natural selection drives evolution, then female fitness should be reduced only for between-population crosses that involve populations using different hosts.

2. MATERIAL AND METHODS

(a) Study system

Timema cristinae were captured from 28 populations in the Santa Ynez Mountains, California in spring 2003 and 2004 using sweep nets. A population is all the insects captured within a homogeneous patch of a single host-plant species. 'Parapatric' insect populations are in contact with a population of insects adapted to the alternative host (i.e. they have an adjacent population using the alternative host). 'Allopatric' populations are separated from all other populations adapted to the alternative host by distances greater than 50 times the 12 m per-generation gene flow distance (Sandoval 1993). Allopatric populations were paired together such that the geographical distance between each population pair was comparable (see electronic supplementary material for a map of the study sites and table 1 for population pairing). Sample sites with both hosts were chosen such that there was only one population on each host species (i.e. each parapatric population had only one adjacent population on the alternative host).

(b) Population crosses

Walking sticks were reared in glass jars at the University of California at Santa Barbara (20 °C) with 10–15 individuals per jar. Individuals from different populations and the sexes were kept separate. Within-population and between-population crosses were conducted (the male and the female from the exact same versus from different populations, respectively).

All individuals used in the crosses were sexually immature first instars captured in the field that were reared to sexual maturity on *Ceanothus* cuttings (about four to six weeks of rearing). Within 2 days of achieving sexual maturity, a single virgin male and a single virgin female were housed together in a Petri dish until copulation was observed and then fed *Ceanothus* cuttings every second day until the female died (females lay eggs singly). We recorded the longevity of females and the number of eggs laid.

Egg number in some broods was recounted in the same year and in the subsequent year, to estimate repeatability (i.e. measurement error). Further statistical analyses were conducted on egg number from the year of initial count, in case some eggs were lost or damaged. This does not affect our results, because egg number was highly repeatable both within ($r=0.96$, $p<0.001$, $n=26$) and between years ($r=0.95$, $p<0.001$, $n=107$). Female longevity was recorded in 652 of the 689 crosses.

Four main types of crosses were examined (within-population, between-populations using the same host, between parapatric populations using different hosts, between allopatric populations using different hosts). Female longevity was correlated with lifetime fecundity ($r=0.75$, $p<0.001$) and the slope of this relationship did not differ among the four cross-types (cross-type \times longevity interaction, $F_{3,652}=0.17$, $p=0.92$; ANCOVA test for homogeneity of slopes). Thus, we calculated oviposition rate as the residuals of a regression of lifetime fecundity on female longevity.

(c) Statistical analyses

ANOVA analyses were used to test whether lifetime fecundity, female longevity or oviposition rate differed among the four main types of crosses examined (within-population, between-populations using the same host, between parapatric

populations using different hosts, between allopatric populations using different hosts). These analyses test for cryptic reproductive isolation, but are not well suited for the comparative test of whether ecological divergence drove evolution because individuals (rather than population pairs) are the unit of replication.

We used three analyses to provide an explicit test of the hypothesis that ecological divergence results in greater cryptic reproductive isolation. First, we used one-tailed t -tests to examine whether the reduction in female fitness for between-population versus within-population crosses was greater for allopatric pairs of populations using different hosts than for allopatric pairs using the same host. Second, a permutation test was used to provide a non-parametric test of the same hypothesis. Third, we analysed the results within each of the three ecological scenarios considered separately (allopatric pairs on the same host, parapatric pairs on different hosts, allopatric pairs on different hosts). Paired t -tests were used to test for significant differences in female fitness for between-population versus within-population crosses, with a separate paired t -test conducted for each of the three ecological scenarios. These paired t -tests still treat pairs of populations within each scenario as the unit of replication, and thus are appropriate for comparing different scenarios.

Two types of further ANOVA analyses were conducted. First, Tukey's *post hoc* tests were used to examine which specific pairwise comparisons between cross-types in the overall ANOVA analyses described above were significantly different from one another. Year (2003 or 2004) was also included as a factor in all analyses reported. Interactions between year and cross-type were always statistically insignificant, and thus this interaction term is not included in the analyses presented. However, retaining this interaction did not affect our conclusions in any way (e.g. main analysis with lifetime fecundity; main effects of cross-type, $F_{3,689}=3.71$, $p<0.05$; cross-type \times year interaction, $F_{1,689}=0.58$, $p=0.63$). Second, we ran separate ANOVA analyses for each of the 14 population pairs to test whether female fitness differed for between-population versus within-population crosses within each individual population pair. These analyses are required to examine which individual pairs contributed most strongly to overall trends. The model included male population, female population and the interaction between male and female population. We are interested primarily in the interaction term, because a significant interaction indicates that female fitness is dependent on which population the male originates from (i.e. her own or not).

Our rearing regime represents a common garden experiment (i.e. all insects reared on *Ceanothus*) such that differences detected between populations likely have a strong genetic component (we cannot rule out a partial role for maternal effects or early environmental effects, but note that the time in the field is very small relative to the duration of rearing in a common environment). However, this design necessitates that about half of the females (i.e. those originating from *Adenostoma*) are reared on their non-native plant. Potentially, female host of origin could thus contribute to variation in female fitness. We conducted three analyses to test for such an effect, all which indicated it did not occur. First, we conducted the overall ANOVA combined among population pairs including an additional term: the interaction between cross-type and female host of origin (*Ceanothus* or *Adenostoma*). If this interaction is insignificant, it indicates

that the effects of cross-type were independent from female host of origin. Second, we conducted the paired *t*-tests, restricting the results to only females originating from *Ceanothus* (thus comparing female fitness in between-population versus within-population crosses when all the females originate from the same host). Third, the ANOVA analyses on individual pairs of populations deal with this concern explicitly, because they include the main effects of female population.

3. RESULTS

Cryptic reproductive isolation was detected: fecundity and oviposition rate was reduced in females used in between-population versus within-population crosses in some cases (figure 1; main effects of cross-type; fecundity: $F_{1,689} = 6.71$, $p < 0.001$; oviposition rate: $F_{1,652} = 15.27$, $p < 0.001$). These effects of cross-type were independent from which actual host, *Ceanothus* or *Adenostoma*, the female originated from (cross-type \times female host interaction; fecundity: $F_{3,687} = 1.68$, $p > 0.15$; oviposition rate: $F_{3,650} = 0.95$, $p > 0.25$). Female longevity did not differ among cross-types, for the results combined ($p > 0.25$) or for individual population pairs (all $p > 0.05$), so is not considered further.

Comparing the results from the different ecological scenarios revealed that ecological divergence promotes the evolution of cryptic reproductive isolation. The reduction in female fitness for between-population versus within-population crosses was significantly greater for allopatric pairs using different hosts than for allopatric pairs using the same host (table 1; $t_4 = 2.45$, 5.20 , $p = 0.035$, 0.004 for fecundity and oviposition rate, respectively, *t*-tests). Moreover, the reduction in female fitness for between-population versus within-population crosses was greater for all three allopatric pairs of populations using different hosts than for any of the three pairs using the same host (for fecundity and oviposition rate). The probability of this pattern arising by chance is $(3!)(3!)/(6!) = 0.05$ for each component of fitness. Based on these *t*-test and permutation analyses, we conclude that there is a statistical association between divergence in host-plant use and the magnitude of cryptic reproductive isolation.

Analyses of population pairs within each ecological scenario confirmed that ecological divergence is required for cryptic reproductive isolation to evolve. Specifically, significant reductions in fecundity and oviposition rate in between-population versus within-population crosses were only observed for pairs of populations using different hosts (table 1 for paired *t*-tests). For example, crosses between allopatric populations using different hosts exhibited significantly lower female fecundity than did within-population crosses ($p < 0.05$ in the paired *t*-test and $p < 0.001$ in *post hoc* ANOVA). Likewise, crosses between populations using different hosts exhibited significantly lower oviposition rates than did within-population crosses, and this pattern occurred for both allopatric and for parapatric pairs (for both scenarios— $p < 0.05$ in paired *t*-tests and $p < 0.01$ in *post hoc* ANOVA). In contrast to the results for different host pairs, there was no evidence for reduced fecundity or oviposition rate in between-population versus within-population crosses when pairs of populations using the same host were

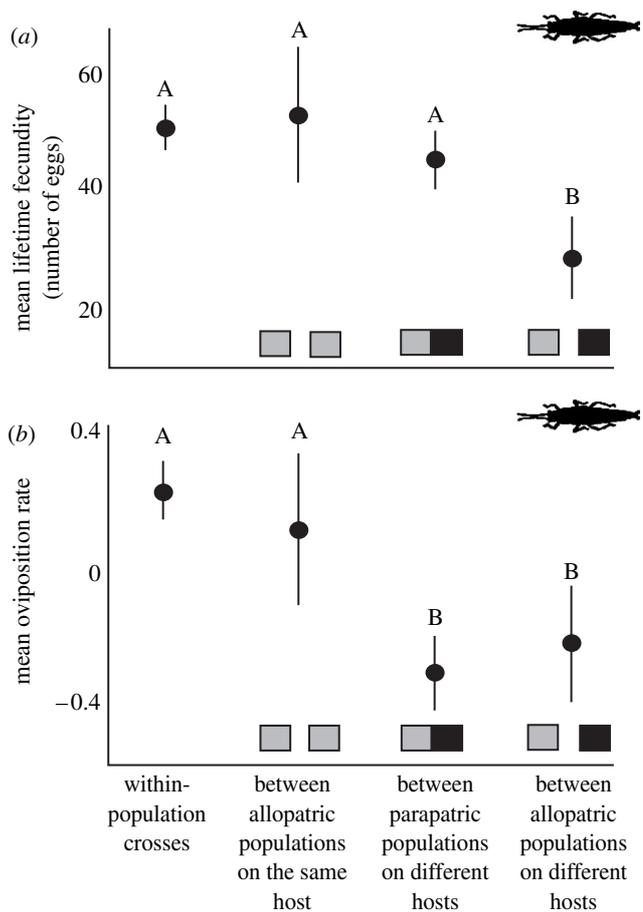


Figure 1. Lifetime fecundity and oviposition rate is reduced for females used in between-population versus within-population crosses, but only when crosses are between populations that use different host-plant species. Differences among the four types of crosses are highly significant ($p < 0.001$, ANOVA). Letters above the 95% confidence intervals denote which specific pairwise comparisons are statistically different from one another in *post hoc* analyses (same letter, no statistical difference; different letters, statistically different at $p < 0.01$). See table 1 for paired *t*-tests and table 2 for analysis of individual population pairs. (a) Lifetime fecundity. (b) Oviposition rate.

examined (all $p > 0.35$ in paired *t*-test and *post hoc* ANOVA analyses; table 1).

The results from ANOVA analyses on individual pairs of populations support the conclusion that ecological divergence drove evolution, and demonstrate that the trends are replicated across populations (table 2). Although differences between individual pairs of populations were not always significant, clear and consistent trends were evident, particularly for allopatric pairs. Female fecundity was significantly reduced in between-population versus within-population crosses for two of the three pairs of allopatric populations using different hosts that were examined ($p = 0.01$, 0.01); the trend in the third population pair was in the same direction ($p = 0.17$), and the overall differences were highly significant when combined among the three population pairs (combined $p < 0.005$). Similar differences were detected for oviposition rate ($p = 0.04$, 0.002 , 0.004 for individual pairs, combined $p < 0.001$). When all the pairs of populations using different hosts are considered (i.e. parapatric populations included), 10 of 11 pairs show reduced oviposition rates for between-population versus within-population

Table 2. Results for individual pairs of populations. (Three distinct types of pairwise comparisons are considered; allopatric pairs on the same host (allop. same hosts), parapatric pairs on different hosts (parap. diff. hosts) and allopatric pairs on different hosts (allop. diff. hosts). Mean fecundity (fecun.), female longevity (long.) and oviposition rate (ovip. rate) is given for each pair as: mean for between-population crosses, mean for within-population crosses. *F* refers to the *F*-ratio from ANOVA analyses testing whether female fitness is dependent on an interaction between male population and female population. Combined probabilities for allopatric pairs of populations on different hosts are <0.005 and <0.001 for fecundity and oviposition rate, respectively. Letter for each population pair refers to the codes used to denote populations in previous studies (Nosil *et al.* 2002, 2003; Nosil 2004). 1, P×PR; 2, PE×WCC; 3, LOG×BT; 4, HVA×HVC; 5, MA×MC; 6, HA×HC; 7, OUTA×OUTC; 8, R12A×R12C; 9, MBOXC×MBOXA; 10, OGC×OGA; 11, VPA×VPAC; 12, LA×VPC; 13, R6C×R23A; 14, SC×LRN.)

pair	fecun.	<i>n</i>	<i>F</i>	<i>p</i>	long.	<i>F</i>	<i>p</i>	ovip. rate	<i>F</i>	<i>p</i>
<i>allop. same hosts</i>										
1	60, 53	62	0.03	0.86	56, 54	0.13	0.72	0.0, -0.1	0.53	0.47
2	44, 54	56	1.70	0.21	39, 47	1.03	0.32	0.3, 0.3	0.48	0.49
3	23, 27	12	0.10	0.77	34, 35	0.00	0.96	-0.5, -0.4	0.40	0.54
<i>parap. diff. hosts</i>										
4	57, 61	85	0.32	0.57	51, 52	0.00	0.99	-0.0, 0.3	1.94	0.17
5	46, 32	32	0.70	0.41	48, 38	1.22	0.28	-0.5, -0.3	1.59	0.22
6	52, 50	16	0.01	0.94	62, 60	0.02	0.90	-0.8, -0.4	0.55	0.47
7	43, 59	52	1.60	0.21	45, 60	4.99	0.03	-0.2, -0.3	0.40	0.53
8	18, 54	70	13.73	<0.001	30, 34	0.65	0.42	-0.5, 0.9	23.98	<0.001
9	50, 56	43	0.04	0.85	55, 49	0.67	0.42	-0.5, 0.2	2.64	0.11
10	44, 28	30	3.07	0.09	54, 37	3.61	0.07	-0.5, -0.4	0.25	0.62
11	44, 48	49	0.15	0.71	51, 50	0.06	0.81	-0.4, -0.1	2.79	0.10
<i>allop. diff. hosts</i>										
12	28, 39	53	1.97	0.17	35, 35	0.00	0.99	-0.3, 0.1	4.47	<0.05
13	25, 44	76	6.46	0.01	26, 30	0.72	0.40	-0.1, 0.6	10.70	<0.01
14	28, 46	53	6.54	0.01	40, 42	0.05	0.82	-0.5, 0.2	10.119	<0.01

crosses (table 2; $p < 0.05$, Binomial test). Conversely, there was no evidence for reduced fecundity or oviposition rate in between-population crosses for any of the three pairs of populations using the same host (all $p > 0.20$; table 2).

4. DISCUSSION

We detected cryptic reproductive isolation between pairs of populations of *T. cristinae* walking sticks, but only between pairs that are ecologically divergent in host-plant use. Thus, ecological divergence promotes the evolution of cryptic reproductive isolation. The proximate mechanism by which oviposition rates are reduced has yet to be elucidated, but several hypotheses exist. First, reduced oviposition rates could reflect reduced fertilization rates with only fertilized eggs being laid (Gregory & Howard 1993). Second, they could reflect the relative inability of foreign seminal proteins to stimulate oviposition (Herndon & Wolfner 1995). Third, females might lower their oviposition rates when sperm is limited, and sperm could be most limited in between-host crosses because it is known that copulation within a 1 h period is least likely in between-host crosses (Nosil *et al.* 2002, 2003). We note that all the females in our experiment copulated at least once (see §2). It is clear that the proximate mechanism, although unknown, is linked to adaptation to different hosts.

Female fecundity is consistently reduced in crosses between allopatric populations using alternative hosts. The results for parapatric pairs are in the same direction, but less definitive and relatively heterogeneous. The observed heterogeneity among population pairs might be expected for sexual forms of reproductive isolation if different traits and different mutations are involved in different populations (Schluter & Price 1993;

Parker & Partridge 1998; Rowe *et al.* 2003). Some specific pairs of parapatric populations exhibited significantly reduced female fecundity in between-population crosses, but most did not (table 2). In contrast to lifetime fecundity, oviposition rates themselves were significantly reduced for crosses between parapatric pairs on different hosts (figure 1; table 1). To the extent that female fitness is reduced in these matings, reductions in female fecundity in between-host matings represent a cost to hybridization that could have facilitated the reinforcement of mating preferences that has been observed in these parapatric populations (Howard & Gregory 1993; Nosil *et al.* 2003).

Unlike mating preferences, cryptic isolation itself is not consistently strongest in parapatry, perhaps because behavioural processes that occur earlier in the life history are more effective at minimizing the costs associated with hybridization (Coyne & Orr 2004). Another explanation involves gene flow, which is known to occur between parapatric populations (Nosil *et al.* 2003). Homogenizing gene flow may have constrained divergence in cryptic reproductive barriers between parapatric populations of *T. cristinae*, as has been observed for other traits, such as colour pattern, body size, body shape, host preference and mate preference (Sandoval 1994a; Nosil *et al.* 2003, in press; Nosil 2004; Nosil & Crespi 2004).

Reproductive isolation is expected to increase with divergence time (Coyne & Orr 2004). However, previous analyses of mitochondrial (cytochrome oxidase I; COI) and nuclear (internal transcribed spacer; ITS) DNA sequence data in *T. cristinae* indicate that differences among population pairs in divergence time are unlikely to account for our results. Two lines of evidence support this claim. First, geographically separated pairs of populations on the same versus different hosts show similar levels of sequence divergence (Nosil *et al.* 2002), yet only the latter

show reduced female fitness in between-population crosses. Moreover, because levels of sequence differentiation are substantial and indicative of long periods of time since divergence (on average 3–4% and 1–2% divergence at COI and ITS, respectively; see Nosil *et al.* 2002, 2003 for details), different population pairs represent relatively independent evolutionary replicates. Second, substantial sequence divergence was detected only for population pairs that were not directly adjacent to one another (Nosil *et al.* 2003), yet such adjacent pairs on different hosts show more evidence for reduced female fitness than do geographically separated pairs on the same host (all the populations from Nosil *et al.* 2002, 2003 are represented in the current study). Thus, ecological divergence in host-plant use, rather than neutral differentiation, predicts cryptic reproductive isolation.

The results of population crosses are not necessarily diagnostic of the existence of sexual conflict (Rowe *et al.* 2003), so we do not claim that sexual conflict does or does not occur in these populations. Rather, our results indicate that if sexual conflict occurs, it must interact with ecological divergence to drive the evolution of reproductive isolation. Some models of speciation via sexual selection include a role for natural selection, but they focus on the evolution of premating isolation (Lande & Kirkpatrick 1988; Schluter 2000). For example, the 'sensory drive' hypothesis predicts that mating signals will involve in correlation with aspects of the environment (Endler 1992). When this occurs, ecologically divergent pairs of populations diverge in mating signals due to an interaction between natural and sexual selection, and show strong sexual (behavioural) isolation as a consequence (Boughman 2002). Sensory drive applies to premating signals, and in fact premating sexual isolation is greater between *T. cristinae* populations using different host species than between populations using the same host (Nosil *et al.* 2002, 2003). Our current findings show that post-mating sexual interactions may also be influenced by the ecological environment.

Most generally, our results demonstrate that even inherently sexual forms of reproductive isolation can evolve as a by-product of ecological divergence. Thus, cryptic reproductive isolation can involve both sexual interactions and ecological divergence (see also Knowles *et al.* 2004). Additional evidence for this hypothesis stems from studies of fertilization success in plants, where cross-fertilization occurs only when style lengths of different species are similar, and style length may be affected by natural selection (Williams & Rouse 1988; Diaz & Macnair 1999). Collectively, these findings show that inclusion of ecology in models of speciation via sexual interactions may lead to novel insights into the evolution of reproductive isolation.

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