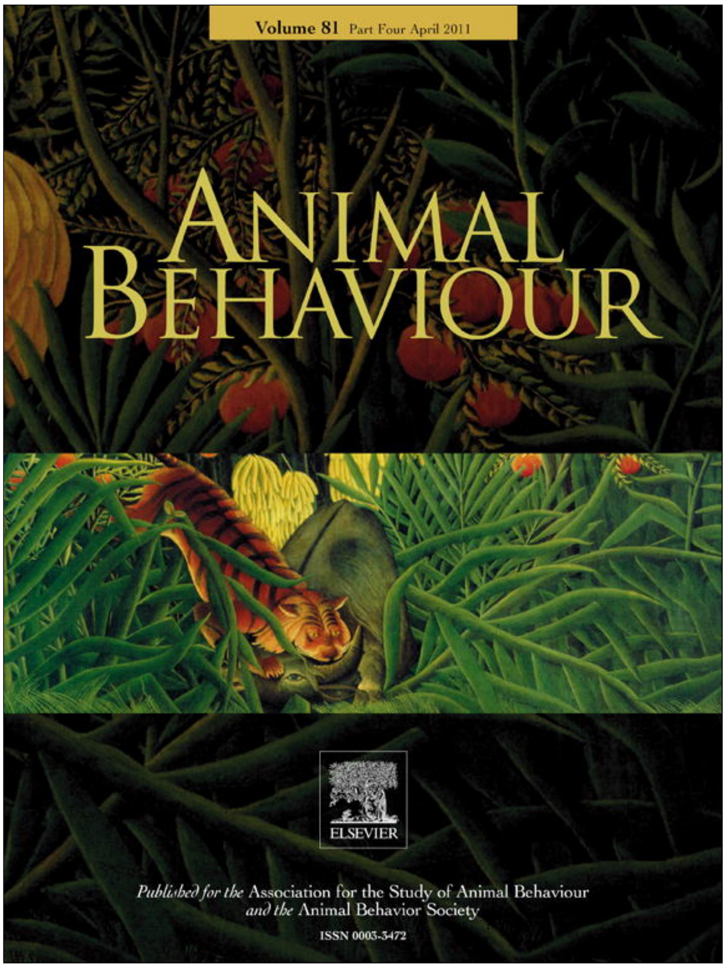
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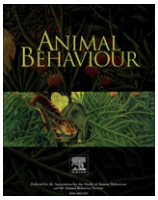

# Host song selection by an acoustically orienting parasitoid fly exploiting a multispecies assemblage of cricket hosts

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Specialization by predators, parasites and parasitoids is often viewed as adaptive because specialization will usually increase search efficiency for suitable hosts (prey). Although many studies have documented interspecific variation in the degree of host specialization, few studies have examined intraspecific variation in specialization among different populations exploiting different numbers of hosts, and even fewer have specifically examined variation in behavioural specialization. Here we examine the acoustic preferences of a parasitoid fly, *Ormia ochracea*, which attacks several *Gryllus* species of field crickets by using the male cricket song to locate hosts. All previous studies with this system have examined fly song preferences in populations of flies that primarily exploit a single host species of cricket. Here we tested a population of flies that naturally parasitizes multiple cricket species. We found significant interspecific host song preferences; however, as predicted, the strength of preference was reduced compared to single-host populations of flies. We also examined fly song selection for intraspecific variants of two of the cricket host species; flies showed significant preference for intraspecific song variants of one of the two cricket species. Our results demonstrate that the degree of behavioural specialization, in a very highly specialized fly, nevertheless shows interpopulation variation consistent with the numbers of host species utilized.

Specialization by predators, parasites and parasitoids on a subset of potential prey or host species is a common feature of coevolutionary arms races (Futuyuma & Moreno 1988; Kaltz & Shykoff 1998). Such specialization may be long-term evolutionary specialization or temporary 'search images' used to enhance efficiency of host (prey) location (Vet et al. 1995). Specialization is thus usually thought of as adaptive; however, there are costs of specialization, both long term (e.g. extinction of the host species dooms an obligate single-host parasite) as well as short term (e.g. specialists may fail to switch to more abundant potentially suitable prey). If there is variation in the degree of specialization, then we might expect different populations to show varying levels of specialization commensurate with numbers of suitable prey species.

Although interspecific comparisons of specialization are common (Stireman & Singer 2003), intraspecific population-level comparisons of behavioural specialization are rare (Robinson et al. 1996). Interspecific variation in specialization may often involve specialist species being derived from more generalist ancestors, as has been found in the seed beetles *Stator limbatus*, a generalist, and *S. beali*, a specialist, evolutionarily derived from within the *S. limbatus* complex (Morse & Farrell 2005). Intraspecific behavioural variation in host plant specialization has been documented among populations of the chrysomelid leaf beetle *Oreina elongata* (Gotthard et al. 2005); however, it remains generally unknown how common is geographically structured intraspecific variation in specialization, and, more specifically, we know very little about how the strength of host discrimination varies with intraspecific variation in specialization among populations.

Parasitoid flies in the genus *Ormia* (Diptera, Tachinidae) are exquisitely specialized parasitoids of acoustically communicating orthopteran insects; gravid female flies locate calling males by eavesdropping on male host-calling song (Cade 1975; Lehmann 2003). Interspecific specialization is well known; for example, *O. depleta* is an acoustic parasitoid of mole crickets, sufficiently host specific to have been imported to the U.S.A. for classical biological control of *Scapteriscus* mole crickets (Frank et al. 1996; Parkman et al. 1996), *O. lineifrons* and *O. brevicornis* are acoustic parasitoids apparently confined to Conocephalinae katydids (Lehmann 2003), and all of the known naturally parasitized host species of *O. ochracea* are New World crickets in the genus *Gryllus*. *Ormia ochracea* has also been introduced to Hawaii (Evenhuis 2003), where it parasitizes the Australian/South Pacific islands' cricket *Teleogryllus oceanicus*, itself introduced to Hawaii (Zuk et al. 1998).

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Everything known to date about *O. ochracea* suggests a very high level of host specialization: the female fly locates male cricket hosts acoustically via an ear specifically tuned to frequencies characteristic of *Gryllus* cricket song, about 4–5 kHz (Robert et al. 1992). The morphological specialization of the ear is remarkable (Robert et al. 1994; Robert & Willi 2000), and is among the smallest highly directional ear currently known (Mason et al. 2001; Arthur & Hoy 2006; Lee et al. 2009). Once female flies locate a potential host cricket, they lay first-instar mobile larvae, which then burrow into the cricket and consume its flesh as they develop over a period of about 10 days, after which time they emerge to pupate, thereby killing their host (Adamo et al. 1995).

What is even more remarkable about *O. ochracea* specialization on *Gryllus* field crickets is that different populations of flies appear to have further specialized behaviourally on locally abundant host species of crickets, such that several populations may be characterized as having a sole or at least a primary local host species of cricket. Across the southern regions of the United States, these primary host species of crickets are *G. rubens*, a nearly exclusive natural host in Florida studied by Walker and colleagues (Walker & Wineriter 1991; Walker 1993), *G. texensis*, a probably exclusive natural host in central Texas studied by Cade, Bertram and colleagues (Cade 1981, 1984; Cade et al. 1996; Gray & Cade 1999; Bertram 2002; Vincent & Bertram 2010), *G. lineaticeps*, a nearly exclusive natural host in coastal southern California studied by Wagner and colleagues (Wagner 1996; Wagner & Basolo 2007; Martin & Wagner 2010), and *T. oceanicus*, a probably exclusive host in Hawaii studied by Zuk and colleagues (Kolluru & Zuk 2001; Lewkiewicz & Zuk 2004; Zuk et al. 2006; Bailey & Zuk 2008b). When each population of flies was presented with a choice between the song of its primary local host and the songs of *Gryllus* species parasitized by the other populations of flies, there was a strong behavioural preference for the song of the primary local host (Gray et al. 2007). An even further degree of specialization has been documented in two single-host populations of flies in coastal southern California and in central Texas, in which flies showed strong preferences for intraspecific song variants within the natural range of variation of their primary local host's song. Flies in coastal southern California prefer *G. lineaticeps* song to alternatives (Gray et al. 2007), but also prefer faster chirp rate variants of *G. lineaticeps* song (Wagner 1996; Wagner & Basolo 2007); similarly, flies in central Texas prefer *G. texensis* song to alternatives (Gray et al. 2007), but also show intraspecific discrimination within *G. texensis* song variants, preferring the species' average numbers of pulses per trill to alternatives with pulses per trill one standard deviation either above or below that of the species' average (Gray & Cade 1999).

Thus, all previous work with this system suggests extraordinary specialization of flies, and their local adaptation to populations of their field cricket hosts. None of the studies conducted to date, however, has examined the responses of flies from populations that naturally exploit multiple host species of crickets. In this paper, we report the results of our studies of such a population of flies from central Arizona, U.S.A. We predicted that such populations would show less interspecific song discrimination among hosts and less intraspecific song discrimination within host species. We also examined the question of why flies show phonotactic preferences in addition to phonotactic responses. Several authors have proposed mechanistic and/or adaptive explanations for song preferences. In our examination of phonotactic responses of flies from a multiple-host population, we found support for some of these ideas and rather less support for others. First, we used field playback experiments broadcasting the species typical songs of four syntopic cricket host species to determine interspecific song preferences of the flies. Second, for two of the four cricket species, we used field playback experiments to examine fly song preferences within the natural variation of those hosts' songs. Third, we use comparative data from this and other studies to examine whether the magnitude of fly interspecific song preference is lower in this population than in populations that exploit a single host species of cricket. Finally, we evaluate various proposals regarding fly song preferences in light of our data.

## METHODS

### *Study Sites and Songs of Local Cricket Hosts*

This study was conducted in the field in Yavapai and Coconino counties, Arizona, U.S.A. The North American *Gryllus* cricket fauna is under extensive revision, and there are many unnamed western U.S.A. species of *Gryllus*, some of which are nevertheless easily diagnosed by combinations of male song, morphology and geography and by DNA sequence analysis (D. B. Weissman & D. A. Gray, unpublished data). In the study area (Fig. 1), five *Gryllus* song types are known natural hosts of *O. ochracea* (D. A. Gray, unpublished host records). Four of the five are clearly distinct species, but none are currently named, and we refer to them here with temporary designations not intended to indicate formal names. We refer to them as 'G15' (an irregular chirping cricket closely related to *Gryllus lineaticeps* Stål and *G. personatus* Uhler), 'Arizona triller' (a uniform trilling species of uncertain taxonomic affinity), 'Arizona stutter-triller' (an erratic stutter-trilling species with very high intra-individual variation in song, possibly closely related to *G. cohni* Weissman), and 'Near integer' (a stutter-trilling species similar to true *G. integer* Scudder but with two sound pulses per chirp within a stutter). The fifth song type we call 'Regular stutter-triller' appears

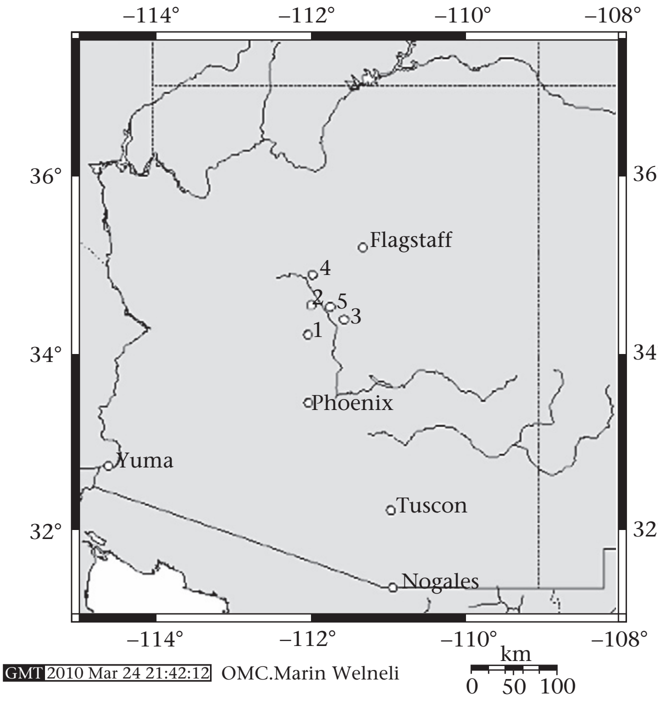

**Figure 1.** Map of the general study area in central Arizona, U.S.A.; major cities and river systems are shown for reference. Numbered circles correspond to playback locations: 1 = Agua Fria National Monument, Bloody Basin Road, Perry Mesa (Yavapai County), 34.23°N, 112.03°W; 2 = Prescott National Forest, Dugas Road (Yavapai County), 34.57°N, 111.99°W; 3 = Coconino National Forest, Fossil Creek Road (Coconino County), 34.41°N, 111.57°W; 4 = Coconino National Forest, Forest Road 525 (Yavapai County), 34.91°N, 111.97°W; 5 = Coconino National Forest, West Clear Creek Road (Yavapai County), 34.55°N, 111.75°W.

similar to Arizona stutter-triller but with almost exclusively three-pulse chirps within a stutter-trill; it is not currently clear to us whether Regular stutter-triller and Arizona stutter-triller are separate species, but we will here refer to each of these song types as a 'species' pending further taxonomic work. Voucher specimens are in the collections of D. A. Gray (California State University Northridge). We tested fly attraction to four of the five song types, which differ greatly in song structure (Fig. 2): G15, Arizona triller, Arizona stutter-triller and Regular stutter-triller.

In our Arizona study area, as well as in Florida, Texas and California, U.S.A., there are other species of *Gryllus* not known to be suitable currently utilized hosts. For example, *G. firmus* in Florida is only occasionally parasitized (Walker & Wineriter 1991), and its song does not attract *O. ochracea* (Walker 1986); univoltine spring adult species such as *G. vocalis* in California are probably safe from *O. ochracea* just due to their phenology; the riparian specialist *G. alogus* in Arizona is not (yet?) a known host of *O. ochracea*. Thus, our study addresses host specialization with respect to utilized hosts, rather than all potential hosts.

### *Characterization of Song Variation*

To create synthetic song exemplars for playback, we needed to first characterize the degree of natural song variation in these four song types. Song recordings were collected and digitally analysed for each cricket species. Both male and female crickets were collected at various localities in Yavapai and Coconino Counties, Arizona, U.S.A. from August through October of 2006; each of these species of crickets is flight-capable, so we considered all crickets of a given species within this area a genetically interconnected population. Field males as well as first-generation laboratory-reared males had their calling songs digitally recorded (16 bit, 44.1 kHz) and analysed using CoolEdit2000® (Syntrillium Software Corporation, Phoenix, AZ). Cricket songs are composed of pulses of sound caused by the closing stroke of the wings, separated by silent intervals corresponding to the opening stroke of the wings. These pulses are arranged into groups that vary in temporal structure and pattern depending on the species. Two of our study species have simple song structures and two have complex song structures. We define our song structure terminology as follows: a long series of sound pulses is called a trill, short groupings of fewer sound pulses are called chirps, and irregular numbers of chirps grouped within a trill produce what we call a 'stutter-trill' song; currently there is no consensus in the literature regarding how many pulses constitute a 'trill' versus a 'chirp'; however, *G. multipulsator*, with 12–16 pulses per song unit, is referred to as a 'chirper' (Weissman et al. 2009), whereas *G. texensis*, with 15–110 pulses, is referred to as a 'triller' (Cade & Otte 2000). The song characters we measured were pulse duration (length of a single pulse of sound, ms), the interpulse interval (silent interval between two pulses of sound, ms), frequency (Hz), the number of pulses per chirp or trill, the interchirp or intertrill interval (the silent interval between the end of one chirp, trill or stutter-trill and the start of next chirp, trill or stutter-trill, ms), the number of chirps grouped within a stutter-trill, the interchirp interval within a stutter-trill (the silent interval between the end of one chirp and the start of the next chirp within a stutter-trill), and temperature (°C).

### *Synthetic Song Construction*

For the interspecific playbacks, we constructed species-typical synthetic songs for all four cricket species in CoolEdit2000® using values taken from the natural song variation analysis (see Results, Table 1). Cricket song sound pulses are not perfectly pure tone and tend to drop in frequency towards the end of a sound pulse (probably due to a slowing down of the closing stroke of the wing), so, for each species separately, we constructed a synthetic sound pulse with a 500 Hz frequency modulation itself modulated at a rate of 5000 Hz, with a first harmonic at 20% amplitude and a second harmonic at 10% amplitude; the frequency of the pulse itself reflected the average peak frequency of the song, which varies by species (see Results, Table 1), and dropped 200 Hz over the duration of the sound pulse; we used an amplitude envelope to shape the sound pulse with a natural rise and fall in peak amplitude. To facilitate field sound-level calibration, all sound pulses for each species had identical peak amplitude, and we created a 5 kHz pure tone of the same amplitude. Then, for each species separately, we copied and pasted the sound pulse with inserted silent intervals, which also varies by species (see Results, Table 1), to create a species-typical calling song and then digitally transferred the

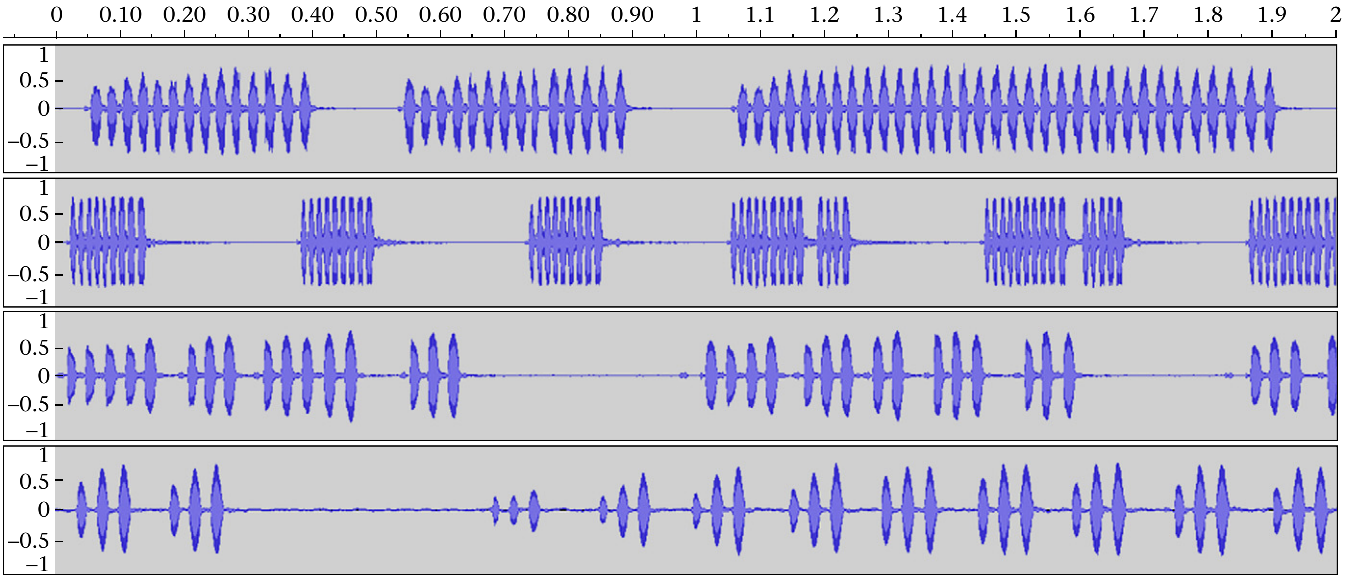

**Figure 2.** Songs of four of the cricket species that are known hosts of *Ormia ochracea* in the study area. Shown (top to bottom) is 2 s of song for the Arizona triller (24.5 °C), G15 (23.8 °C), Arizona stutter-triller (25.9 °C) and Regular stutter-triller (23.3 °C).

**Table 1**
Song variation in four *Gryllus* hosts of *Ormia ochracea* in central Arizona, U.S.A.

| Species | *N* | Pulse duration (ms) | Interpulse interval (ms) | Peak frequency (kHz) | Pulses/chirp or trill | Interchirp or intertrill interval (ms) | Number of chirps/ stutter-trill | Interchirp intervals/ stutter-trill (ms) | Temperature (°C) |
|---|---|---|---|---|---|---|---|---|---|
| G15 | 17 | 7.4±1.1 (7) | 3.8±1.6 (4) | 5.5±0.36 (5.5→5.3) | 8.2±0.8 (8) | 268±62 (268) | – | – | 24.8±2.4 |
| Arizona triller | 18 | 14.4±1.6 (14.5) | 8.5±2.4 (8) | 4.5±0.25 (4.5→4.3) | 39.0±21.0 (33) | 255±111 (220) | – | – | 26.0±1.6 |
| Arizona stutter-triller | 9 | 19.9±3.9 (20) | 19.4±19.1 (20) | 4.8±0.45 (4.9→4.7) | 4.4±1.2 (4.9±2.4) | 338±224 (280) | 6.1±3.7 (6.4±1.1) | 40.0±11.4 (40) | 24.5±1.9 |
| Regular stutter-triller | 18 | 20.0±4.5 (20) | 9.8±2.9 (10) | 4.8±0.24 (4.9→4.7) | 3.0±0.6 (3) | 393±335 (400) | 7.8±3.7 (8) | 33.9±10.0 (35) | 25.2±2.0 |

Values are means ± SD; values used for synthetic songs are given in parentheses.

synthetic songs to a CD-R for playbacks in the field. Note that for the Arizona stutter-triller, we constructed a synthetic song with variation in the numbers of pulses per chirp and in the numbers of chirps per stutter-trill as this species has extreme intraindividual variation in these song characters and we considered it important that our species-typical song should reflect this high level of intraindividual variation; the other species show far less variation within individuals.

Synthetic songs for the intraspecific song playbacks were created in the same manner. For G15, we created song variants that differed in chirp rate by varying the interchirp interval to produce four songs: one with an interchirp interval one standard deviation above the average (330 ms interval, net chirp rate 2.41 chirps/s), one with the average interchirp interval (268 ms interval, net chirp rate 2.84 chirps/s), one with an interchirp interval one standard deviation below the average (206 ms interval, net chirp rate 3.44 chirps/s), and one with an interchirp interval two standard deviations below the average (144 ms interval, net chirp rate 4.38 chirps/s). For Regular stutter-triller intraspecific variants, we manipulated the numbers of chirps within a stutter-trill. We created four song variants that had either 4, 8, 12 or 16 chirps per stutter-trill, representing −1 SD, the mean, +1 SD or +2 SD, respectively. For both G15 and Regular stutter-trillers, we chose to manipulate these song characters, rather than others, because they showed high natural intraspecific variability and greatly affected total acoustic output of song, and in other *Gryllus* species, they are known to be both condition dependent and subject to directional selection via female choice (i.e. chirp rate in G15 and *G. lineaticeps*: Wagner 1996; Wagner & Hoback 1999; Wagner & Basolo 2007; chirps/stutter-trill in Regular stutter-trillers and calling bout duration in true *G. integer*: Hedrick 1986, 2005; Hedrick & Weber 1998).

### *Song Playbacks*

Interspecific song playback experiments were conducted in the field for 10 nonconsecutive nights from August to October 2007, 2008 and 2009. Intraspecific song playbacks of G15 song variants were conducted for 10 nonconsecutive nights between the August and October 2008 and 2009; intraspecific song playbacks of Regular stutter-triller song variants were conducted for 26 nonconsecutive nights from August to October 2007, 2008 and 2009. Synthetic songs were broadcast in clearings at five field sites in Arizona (Fig. 1). At each field site, four Altec Lansing® VS2320 audio speakers (facing skywards) were placed in a square formation 3 m per side. A screen mesh flytrap (Walker 1989) was then placed over each speaker to capture attracted flies. The synthesized songs were played simultaneously using Sony® D-EJ100 CD Walkman players; songs were broadcast at 80 dB at 1 m above the speaker (Radio Shack 33-2055 meter, dB re. 20 μPa). The order in which any given speaker played the synthetic song of a particular species was assigned haphazardly each night. Song playbacks began at dusk (ca. 1930 hours), and ended 3 h later (ca. 2230 hours). Continuous broadcast of song is a useful technique for measuring the relative attraction of flies to a particular song type; however, it greatly exaggerates the risk of parasitism for real crickets, which typically do not call for 3 h continuously (Cade 1984). Flies that were either on or in a particular flytrap were scored as having a preference for that song. At the end of a particular night, sometimes the captured flies were retained for other studies, but most were released. As our playbacks were conducted on nonconsecutive nights across five field sites, over 3 years, we consider pseudoreplication of data via recapture of individual flies highly unlikely.

### *Strength of Fly Host Song Preference in Single- versus Multiple-host Populations*

To test whether the strength of fly attraction differs in our multiple-host population of flies from that in various single-host populations of flies, we compared the percentage of flies attracted to the most preferred song between six single-host populations ($N = 16$ replicate sets of playbacks; data from Gray et al. 2007) and our multiple-host species population ($N = 10$ replicate sets of playbacks; data from this study). The percentage of flies attracted to the most preferred song was used as a metric of unanimity among flies in their preference as it is a simple measure that captures the essence of a population-level preference, although it does not necessarily reflect an individual fly's strength of preference.

## RESULTS

### *Natural Song Variation*

Table 1 presents data on natural song variation in these four cricket species; parameters for our synthetic songs are also shown. As field playback temperatures were within the range of our recording temperatures, no attempt was made to correct for the minor differences in temperature among recordings.

### *Interspecific Song Selection by Flies*

A total of 378 flies were caught over the 10 nights of playback (Fig. 3). Flies showed significantly nonrandom song selection (goodness of fit test: $G^2_3 = 40.45$, $P < 0.0001$). Adjusting statistically for multiple post hoc comparisons (see Box 17.5 in Sokal & Rohlf 1995), pairwise comparisons of fly responses differed between Regular stutter-triller and Arizona triller (simultaneous test procedure: $G^2 = 21.31$, $P < 0.001$), Regular stutter-triller and Arizona stutter-triller ($G^2 = 26.24$, $P < 0.001$), G15 and Arizona stutter-triller ($G^2 = 9.5$, $P < 0.001$) and G15 and Arizona triller ($G^2 = 6.60$, $P < 0.05$), but not between Regular stutter-triller and G15 ($G^2 = 4.24$, $df = 1$, $P = 0.237$) or Arizona stutter-triller and Arizona triller ($G^2_1 = 0.26$, $P = 0.606$). Thus, the overall attractiveness of each song was G15 = Regular stutter-triller > Arizona triller = Arizona stutter-triller.

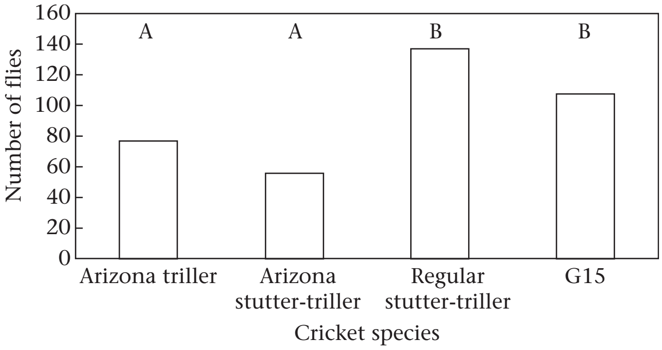

**Figure 3.** Numbers of flies caught at each of the four host songs over 10 replicate nights of playbacks. Different letters indicate statistically significant differences between hosts.

### *Intraspecific Song Selection by Flies*

G15 intraspecific playbacks caught 161 flies over 10 nights. Fly preference differed significantly between the four song variants ($G^2_3 = 52.70$, $P < 0.0001$), seeming to increase linearly with chirp rate (Fig. 4).

For the Regular stutter-triller song playbacks, 509 flies were caught over 26 nights. Flies showed no significant preferences among the four song variants ($G^2_3 = 7.43$, $P = 0.06$), with no apparent trend in attractiveness across songs (Fig. 5).

### *Strength of Fly Host Song Preference in a Single- versus Multiple-host Populations*

Table 2 presents the data used to test whether the population-level preference of flies for particular hosts' songs differs between single-host and multiple-host populations. In each of the playback replicates, four songs of different known *O. ochracea* hosts were broadcast simultaneously; thus, the null expectation of fly attraction to any given song was 25% for all replicates. Shown is the percentage of flies caught at the most preferred song. In the fly populations specializing on a single host species ($N = 16$ sets of playback replicates), the most preferred song attracted 59.75 ± 15.75% (mean ± SD) of responding flies, whereas in our central Arizona multiple-host population, 43.50 ± 4.65% (mean ± SD) of flies were attracted to the most preferred song ($N = 10$ sets of playback replicates). There was a significant difference in fly attraction depending on the type of host species assemblage (ANOVA on arcsine square-root transformed data: $F_{1,24} = 8.97$, $P = 0.006$).

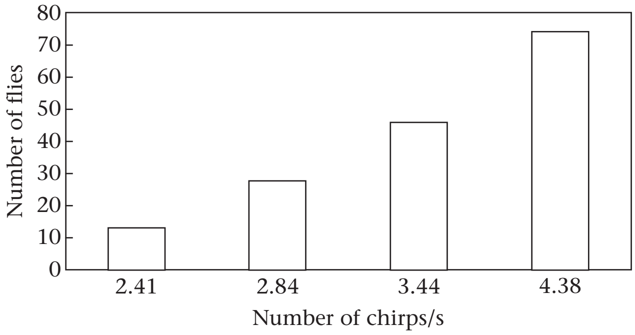

**Figure 4.** Numbers of flies caught over 10 replicate nights of playbacks of four G15 song variants with increasing chirp rates.

**Figure 5.** Numbers of flies caught over 26 replicate nights of playbacks of four Regular stutter-triller song variants with increasing numbers of chirps per stutter-trill.

## DISCUSSION

The most novel result of this study is the demonstration that the strength of fly preference among host songs depends on the type of host species assemblage. The strength of fly preference among potential host songs was greater for fly populations that specialize on a single host species than it was for our central Arizona fly population, which exploits multiple host species. Such interpopulation variation in the degree of behavioural specialization is a relatively novel finding, and is clearly consistent with our predictions. On the other hand, our results for fly preferences based on intraspecific variation in host song were less clear-cut: flies showed no preference for Regular stutter-triller song variants, but showed a clear preference for G15 song variants with faster chirp rates. We predicted weak or nonexistent intraspecific song preference in this population of flies given that they have several host species to choose from. The fact that they nevertheless showed a strong

**Table 2**
Data used to test for differences in population-level preference of flies for particular hosts' songs in single-host and multiple-host populations

| Host assemblage | Locality | % Flies at most preferred song | Source |
|---|---|---|---|
| Single species | Florida | 50 | Gray et al. 2007 |
| Single species | Florida | 62 | Gray et al. 2007 |
| Single species | Florida | 89 | Gray et al. 2007 |
| Single species | Florida | 60 | Gray et al. 2007 |
| Single species | California | 67 | Gray et al. 2007 |
| Single species | California | 42 | Gray et al. 2007 |
| Single species | California | 67 | Gray et al. 2007 |
| Single species | California | 72 | Gray et al. 2007 |
| Single species | California | 52 | Gray et al. 2007 |
| Single species | California | 35 | Gray et al. 2007 |
| Single species | California | 40 | Gray et al. 2007 |
| Single species | California | 56 | Gray et al. 2007 |
| Single species | Texas | 51 | Gray et al. 2007 |
| Single species | Texas | 61 | Gray et al. 2007 |
| Single species | Texas | 60 | Gray et al. 2007 |
| Single species | Hawaii | 92 | Gray et al. 2007 |
| Multiple species | Arizona | 40 | This study |
| Multiple species | Arizona | 39 | This study |
| Multiple species | Arizona | 53 | This study |
| Multiple species | Arizona | 49 | This study |
| Multiple species | Arizona | 44 | This study |
| Multiple species | Arizona | 45 | This study |
| Multiple species | Arizona | 38 | This study |
| Multiple species | Arizona | 44 | This study |
| Multiple species | Arizona | 42 | This study |
| Multiple species | Arizona | 41 | This study |

In each playback replicate, four songs of different known *O. ochracea* hosts were broadcast simultaneously; thus, the null expectation for any given song was 25%. Shown is the percentage of flies caught at the most preferred song.

preference for faster G15 chirp rates is interesting, and parallels song preferences in coastal southern California flies that attack *G. lineaticeps* (Wagner 1996; Wagner & Basolo 2007); recall that G15 and *G. lineaticeps* are close relatives with similar song structures.

It could of course be the case that these different host-specific populations of flies are in fact host-differentiated cryptic species, as has been found with other parasitoids (Smith et al. 2006, 2007). Mitochondrial DNA sequences of *O. ochracea* from Florida, Texas, Arizona, California and Hawaii, U.S.A., as well as Sonora, Mexico, suggest that all studied *O. ochracea* populations belong to the same species (D. A. Gray, unpublished data); thus, the local adaptation of flies to populations of their field cricket hosts appears to be due to intraspecific geographically structured specialization.

Why do flies show acoustic preferences? The results of this study provide an opportunity to examine different proposals regarding the basis and perhaps selective value of song preferences within this fly. Our results show that G15 and Regular stutter-triller attracted significantly more flies than did Arizona triller or Arizona stutter-triller. Here we will review two mechanistic hypotheses, three adaptive hypotheses, and one historical hypothesis. One type of mechanistic explanation is that an intrinsic song template constrains flies' responses to certain song types (Müller & Robert 2002). Hearing sensitivity almost certainly does constrain *O. ochracea* to cricket hosts with songs in the 4–5 kHz range typical of *Gryllus* (Ramsauer & Robert 2000), but it is hard to imagine a constraining song template with respect to variation in the temporal properties and arrangements of cricket sound pulses: variation in song among the four cricket species studied here, as well as among the previously studied host species *G. rubens*, *G. texensis*, *G. lineaticeps* and *T. oceanicus* is very extensive. Pulse durations of these eight host species (at ca. 25 °C) range from a low of 7–9 ms (in *G. lineaticeps*, *G. rubens*, *G. texensis* and G15), up to about 45 ms in *T. oceanicus*. Pulse rates range from about 12/s in the long-chirp portion of *T. oceanicus* song up to nearly 90/s in G15. Song structure ranges from long trills (*G. rubens*, *G. texensis* and Arizona triller) to chirps (*G. lineaticeps* and G15) to complex stutter-trills composed of chirps with either low intraindividual variation in pulse number (Regular stutter-triller) or high intraindividual variation in pulse number (Arizona stutter-triller). Thus, both the data presented here and previous work (Müller & Robert 2002; Gray et al. 2007) suggest that *O. ochracea* is extremely tolerant of wide variation in song structure, inconsistent with a constraining song template.

An alternative mechanistic explanation for song preferences is a 'psychoacoustic' effect that affects the ability of flies to localize song (Müller & Robert 2002); psychoacoustic effects are the joint property of physical sound waves and the perceptual system, which affect sound perception and localization. If this is an important phenomenon in *O. ochracea*, then we might predict that songs with more acoustic power would be preferred over songs with less acoustic power. At the interspecific level of comparison among hosts' songs, this explanation also seems unlikely. Two features of songs (other than amplitude) might contribute most to a psychoacoustic preference: total sound output or numbers of signal onsets/offsets. Of the four species studied here, the Arizona stutter-triller has the most total sound output, approximately 526 ms of sound for each second of song, followed by the Arizona triller, with about 455 ms sound/s song, then the Regular stutter-triller (419 ms sound/s song), and finally G15, with only about 112 ms sound/s song. As the Regular stutter-triller and G15 attracted the most flies, but had the least sound output per unit time, total sound does not seem to predict fly attraction. Sound onsets/offsets may be a more important psychoacoustic stimulus. If so, we would predict that Regular stutter-trillers and Arizona stutter-trillers would attract the most flies; Regular stutter-trillers did attract high numbers of flies, but Arizona stutter-trillers did not. Previous results (Gray et al. 2007) also argue against a strong psychoacoustic effect: *G. rubens*, *G. texensis*, *G. lineaticeps* and *T. oceanicus* songs vary greatly in presumed psychoacoustic stimulus power, yet no one song was consistently preferred by all flies; rather, flies preferred the host song typical of their own population. Thus, we agree with Müller & Robert's (2002) conclusion that neither strictly mechanistic explanation seems strongly supported.

Three broadly adaptive explanations of fly song preferences are worth considering in light of our results: host size, host quality and host abundance. First, if song reflects host size, then it may be advantageous for flies to prefer song variants associated with larger-sized hosts. This is because fly pupal mass, and hence adult size, depends upon the quantity of resources per larvae (Adamo et al. 1995; Kolluru & Zuk 2001). In our assemblage of four host cricket species, if flies preferred the songs of larger crickets, then we would expect flies to have ranked these four hosts as follows: G15 > Arizona triller > Regular stutter-triller ≥ Arizona stutter-triller. Second, if song reflects host qualities other than size, such as nutritional condition, then we might expect flies to prefer faster, more energetically expensive songs (Wagner 1996). Our interspecific song preference data do not support this as Arizona stutter-trillers clearly had the highest-energy song but attracted relatively few flies; interspecific comparison may not be the most appropriate level of analysis for effects of nutritional condition on song, however. Our intraspecific song selection data were split: G15 song with faster chirp rates did attract significantly more flies than did G15 song with slower chirp rates. Chirp rate in the closely related *G. lineaticeps* is known to reflect male nutritional condition (Wagner & Hoback 1999; Wagner & Reiser 2000). However, Regular stutter-triller song with more chirps per stutter-trill did not attract more flies than Regular stutter-triller song with fewer chirps per stutter-trill. Our 'chirps per stutter-trill' may well be a condition-dependent trait as it is acoustically equivalent to calling bout duration in *G. integer*, which is known to be condition dependent (Hedrick 2005). Third, relative host abundance may affect fly song preference. If all potential host species are approximately equally suitable hosts, and fly fitness is host limited, then we might expect flies to adopt search strategies focused on abundant, recently encountered host songs. This explanation mirrors that given by Gray & Cade (1999) for *O. ochracea* in Texas that preferred the average numbers of pulses per trill in *G. texensis* song; preference for abundant song types should minimize fly search costs. Although we did not formally quantify cricket species abundance in our study area, we can approximately rank the relative abundance of three of these species as G15 = Arizona stutter-triller > Arizona triller. These rankings are only our subjective impressions based on numbers of calling males heard, and we have not included an estimated abundance rank for Regular stutter-triller because it is sometimes difficult for us to distinguish it from Near integer in the field by ear alone. If relative host abundance does prove to be an important predictor of fly song preferences in multihost assemblages, this suggests that flies retain information on host abundance longer than the 3 h playback protocol that we used. This is because our playback protocol simulated equal abundance and calling effort of hosts, yet flies nevertheless distinguished among the playbacks. Experience is known to affect song preferences in some invertebrates (Bailey & Zuk 2008a); however, the temporal duration of such an effect is generally unknown (Dukas 2008). In summary, although predictions from each of these three adaptive explanations at least partially match our data, our data are not even close to a rigorous test of these ideas and this is clearly an area in need of future work.

The final possibility we consider is a historical adaptive explanation. By this we mean that current song preferences may reflect prior adaptation rather than current adaptation. For example, if the ancestral lineage of *O. ochracea* had adaptively specialized on song types similar to those of current G15 and Regular stutter-triller, then present-day song preferences may be a hold-over from past specialization. The *Ormia* lineage is New World in origin, and ormiines in general peak in diversity at tropical latitudes (Lehmann 2003). Neither the current nor the ancestral range of *O. ochracea* is known with certainty; examination of museum specimens from the New World Tropics reveals many mislabelled specimens. *Ormia ochracea* is known from across the southern United States, northwestern Mexico, and a single specimen has been confirmed (by DNA sequence) from Guanacaste, Costa Rica; a close relative within the genus, *O. depleta*, is native to Brazil. This collectively suggests that the ancestral lineage of *O. ochracea* may have spread northwards into the southern United States. This is potentially informative as G15 is distributed throughout the Sonoran desert region, south to northern Sinaloa, Mexico. Thus, G15 type song is likely to have been encountered by ancestral *O. ochracea*; Arizona stutter-triller has a similar distribution, south at least to southern Sonora, Mexico. The problem we see with this type of historical adaptive explanation is that it basically envisions a constraining song template, where that song template is the product of past selection rather than nonadaptive neurophysiology. The argument against a constraining song template, given above, applies equally well to a song template based on past selection as it does against a constraining song template based on nonadaptive mechanistic constraint.

Here we have shown a degree of intraspecific variation in the behavioural specialization of *O. ochracea*, generally considered a highly specialized species. The variation we document is in a direction consistent with our predictions of enhanced specialization in single-host populations as compared to a multiple-host population. Clearly this species of fly retains significant flexibility in its search strategy; even the single-host populations are not exclusively attracted to their own host species' song. Such flexibility may have been instrumental in allowing this species of fly to broaden its geographical range, and perhaps was prerequisite in allowing exploitation of a completely novel host, *T. oceanicus*, in Hawaii. Intraspecific variation in behavioural specialization may commonly reflect the numbers of suitable resources available in any given environment. We expect that intraspecific variation in the degree of host specialization may be more common than is generally apparent from the literature. Few other such examples exist. In the only other directly analogous study we know of, different populations of the nest parasitic bird the common cuckoo, *Cuculus canorus*, showed no specialized local adaptation to different host populations of their reed warbler, *Acrocephalus scirpaceus*, hosts (Aviles et al. 2011). Clearly, a great deal more work remains to be done to link local adaptation and intraspecific variation in degree of specialization. The degree of specialization may be anticipated to reflect the relative benefits of specialization, such as increased efficiency of host location, that trade-off against the limitations imposed by such specialization.

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