ORIGINAL ARTICLE

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Latency to resume calling after disturbance in the field cricket, *Teleogryllus oceanicus*, corresponds to population-level differences in parasitism risk

Abstract A possible parasitoid-evasion behavioral adaptation is examined in male field crickets, *Teleogryllus oceanicus*, from three Hawaiian islands where parasitoid prevalence varies naturally among islands. *Ormia ochracea*, the parasitoid fly that parasitizes *T. oceanicus* on these islands, uses male calling song to locate its hosts. We used laboratory-reared males from three Hawaiian islands to determine if there are population differences in the time it takes for calling males to resume calling after a standardized disturbance. Males follow the expected pattern; males from the island with the greatest risk of parasitism have the longest latency to resume calling, and males from the island with the least risk of parasitism have the shortest latency to resume calling. Results are discussed in the context of behavioral adaptations to differing parasitism levels, and trade-offs between natural and sexual selection.

Keywords Parasitoid · *Teleogryllus oceanicus* · *Ormia ochracea* · Risk aversion · Calling song

Introduction

Many aspects of reproduction have been shown to increase individuals' risk of predation and/or parasitism (Endler 1987; Magnhagen 1991; Forsgren and Magnhagen 1993; Zuk and Kolluru 1998). For acoustically signaling animals, such as crickets, in which males call to attract mates, this trade-off between survival and reproduction can be particularly important, since such calls, by their very nature, must be conspicuous (Cade 1975; Bell 1979; Gray and Cade 1999; Kolluru 1999; Lehmann et al.

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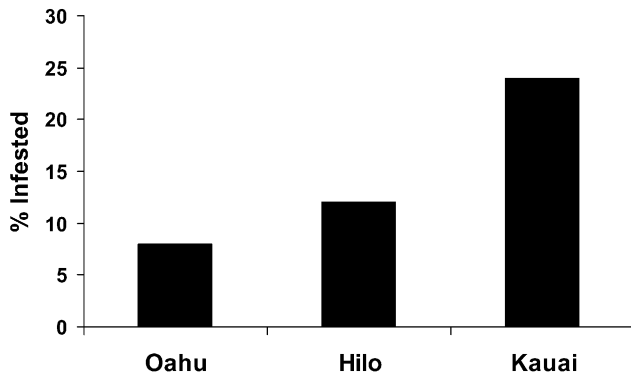Tel.: +1-909-8277021
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2001). In such cases, we expect to find selection for behaviors that allow for increased reproductive effort during times of low predation risk and decreased reproductive effort during times of high predation risk. These behavioral adaptations must entail early detection and evasion of predators (Miller and Surlykke 2001). Such behaviors may involve increasing the use of cover when risk is high (Banks 2001), or using indications of predator proximity as cues to cease displaying (Spangler 1984; Jennions and Backwell 1992).

The field cricket *Teleogryllus oceanicus* was introduced to three of the Hawaiian islands (Oahu, the Big Island of Hawaii, and Kauai) at least 150 years ago (Kevan 1990; Zuk et al. 1993). Native to Australia and the Pacific, *T. oceanicus* is subject to parasitism in Hawaii by the introduced parasitoid fly, *Ormia ochracea* (Zuk et al. 1993, 1998). *O. ochracea* females home in on the calling song of *T. oceanicus* and deposit larvae on and around the cricket (Cade 1975). These larvae burrow into the body of the cricket and feed on thoracic and abdominal muscle tissue for 7–10 days, after which they emerge from the body cavity of the cricket, killing it within hours (Adamo et al. 1995). Previous work has documented several responses to parasitism in *T. oceanicus*, including changes in song structure, the diel distribution of calling, and the age structure of parasitized populations (Zuk et al. 1993; Simmons and Zuk 1994; Rotenberry et al. 1996). The three islands differ in the prevalence of fly infestation, with about 8%, 12%, and 25% of calling males harboring larvae on Oahu, the Big Island of Hawaii, and Kauai, respectively (Zuk et al. 1993; Fig. 1). The prevalences have remained stable over repeated sampling in different seasons and years (Zuk, unpublished data). These differences in the likelihood of becoming parasitized are reflected in the degree of change in the song from unparasitized populations (Rotenberry et al. 1996).

Here we examine another possibly adaptive response to degree of risk: the latency to resume calling after a disturbance. Hedrick (2000) demonstrated that call latency was an effective measure of caution for another field cricket with varying degrees of intrinsic risk. Puffs of air

Fig. 1 Prevalence of *Ormia ochracea* larvae in calling male *Teleogryllus oceanicus* from Kauai, Oahu, and Hilo on the island of Hawaii. Details of cricket and parasitoid collection in Zuk et al. (1993, 1995). Number of males examined: 79 for Kauai, 118 for Hilo, and 126 for Oahu. Kauai samples were collected in 1993 and 1995, Hilo samples in 1992 and 1993, and Oahu samples in 1993 and 1994

were used as disturbances because: (1) these have been shown to elicit escape responses in crickets (Bentley 1975; Gnatzy and Kämper 1990; Gras and Hörner 1992; Kanou 1999), and (2) crickets' cercal wind receptors are extremely sensitive (Kumagai et al. 1998), making it possible that crickets could use such a disturbance in the early detection of approaching parasitoid flies. Crickets are capable of detecting and acting evasively toward the airborne disturbances created by the wing beats of a flying parasitoid wasp (up to 3 cm away) (Gnatzy and Heußlein 1986; Gnatzy and Kämper 1990). *O. ochracea* have a fairly straight flight path and land, on average, 8.2 cm away from their target, although many will land directly on their target (Müller and Robert 2001). This means that crickets may have the opportunity to detect flying natural enemies.

Cessation of calling has been described as an anti-predator startle response in other orthoptera (Sales and Pye 1974; Spangler 1984; Faure and Hoy 2000). It is likely that using silence as an avoidance mechanism is a useful tool for evading parasitism in Hawaiian *T. oceanicus*, since *O. ochracea* are extremely adept at localizing cricket song (Mason et al. 2001). Previous studies indicate that *O. ochracea* are capable of locating sources of cricket calling song even if such calls are fragmented (Müller and Robert 2002) or if calls cease after flies have initiated search flight (Müller and Robert 2001). Importantly, even though flies show such extraordinary homing capabilities, their preference and accuracy at locating call sources diminishes with increasing call fragmentation and periods of silence (Müller and Robert 2001, 2002). Thus, inserting such periods of silence into calling songs after the detection of a possible danger cue is potentially a critically important behavior for male crickets exposed to fly parasitism. However, periods of silence likely reduce males' chances of attracting mates, as female field crickets use calling song to locate males, and they tend to prefer calls of long duration (Wagner

1996) and few interruptions (Hedrick 1986). Thus, while male populations on all islands should face sexual selection pressures to minimize periods of silence, they should also face opposing natural selection pressures imposed by *O. ochracea* to increase periods of silence after a disturbance. The strength of these natural selection forces should vary in accordance to the risk of parasitism experienced by male crickets on each island. We therefore predicted that the latency to resume calling after a disturbance would correspond to the level of parasitism rates among the three islands. Even if *T. oceanicus* males cannot specifically detect approaching *O. ochracea*, we would still expect males from populations exposed to high risk to display a higher degree of general caution than males from lower-risk populations when exposed to risk signals. Since air disturbances cause escape responses in crickets (Bentley 1975; Gnatzy and Kämper 1990; Gras and Hörner 1992; Kanou 1999), it is likely that males from high-risk populations will respond more cautiously (with longer latencies to resume calling) than males from lower-risk populations.

Methods

All males were taken from laboratory stocks that were collected from Oahu, Kauai, and Hilo on the Big Island of Hawaii between 1993 and 1999. New individuals were added to all stocks every 1–2 years to minimize the effects of inbreeding. Details of collection sites are given in Zuk et al. (1993, 2001). Prior to isolation, crickets were housed in mixed-sex and mixed-age containers in 30°C incubators on a 12:12 light:dark cycle. Crickets were given ad libitum water and Flukers Cricket Feed and provided with cardboard egg crates for shelter. Males were isolated in separate containers (8.5 cm in diameter) at least 12 h before use. Isolated males were given cat food and water ad libitum, along with a piece of cardboard egg crate for shelter during the light cycle when experiments were not in progress. Trials took place between 2 and 12 h after the onset of the dark cycle. Cardboard egg crates were removed from all isolated male containers before the onset of trials.

Trials were performed blind, with the identity of the population being tested unknown until after the test was complete. Single individual male containers were placed within Styrofoam boxes to prevent visual disturbances. A turkey baster (a kitchen tool with a rubber bulb on one end of a plastic tube) was attached to the outside of the Styrofoam box and was used to generate a puff of air as a standardized disturbance. The puff of air was transferred into the individual male's container via a 30-cm piece of plastic tubing that connected the plastic tube of the baster to the inside of the male's container.

Males called continuously (with no breaks longer than 2 s) for at least 2 min before a puff of air was delivered into the container by gently depressing the bulb on the baster. In all but one case, the male immediately stopped calling after the puff of air was delivered. The latency for males to resume calling continuously, after this cessation, was timed with a stopwatch. A male was considered to have resumed calling continuously if he called for at least 1 min with no longer than 2-s breaks in the call. If a male did not resume calling within 600 s, he was assigned a conservative latency score of 600 s.

Males were measured three times. Hilo and Kauai males were tested in an environmental growth chamber at 28.4°C. This chamber was unavailable for the Oahu males, which were tested in a room lacking temperature regulation with temperatures varying from 20.0°C to 28.3°C (mean=24.7°C). Although all males were not tested simultaneously, this should not present a problem since

T. oceanicus are not seasonal, and our adult lab colonies are continuously replenished with newly molted adults. Thus, colonies consistently have a mix of young and old adults. Low temperatures could decrease calling activity. If this were the case, we would expect low temperatures to increase latencies to resume calling in Oahu males. However, a Kendall rank-order correlation showed no significant association between temperature and latency to call in Oahu males ($\tau=0.46$, $P=0.32$). Furthermore, any association between latency to call and temperature would only make our comparison of latency scores across populations more conservative, since we predicted that Oahu males should have the shortest latencies to call, and cooler temperatures should tend to increase their latencies. Other studies indicate that low temperatures tend to alter various call components (such as chirp duration, chirp rate, pulse length, pulse rate, and wing stroke rate) in such a way that reduces general call activity (Prestwich and Walker 1981; Pires and Hoy 1992; Ciceran et al. 1994; Martin et al. 2000).

A Jonckheere test for ordered alternatives (Siegel and Castellan 1988) utilized only one measurement per individual; the shortest latency was used for males in all populations. Shortest latencies were used to minimize any slight affects of temperature that may have been experienced by Oahu individuals. Note that the Jonckheere test for ordered alternatives can be used in situations similar to those in which one would use a Kruskal-Wallis rank ANOVA. The difference is that, while a Kruskal-Wallis tests the hypothesis that one of k independent samples is different from at least one other, the Jonckheere test for ordered alternatives is used when one has an a priori hypothesis about the order of sample medians (Siegel and Castellan 1988), such as a test of dosage dependence effect of a drug. Similarly, we had an a priori hypothesis about the order of the population medians. The P-value associated with the Jonckheere test reflects the probability not only that there are significant differences among populations, but also that these differences occur in the predicted order. A chi-square goodness of fit analyses was performed that utilized all three observations per individual.

Results

Males did not differ in their degree of call fragmentation [measured as the number of short (<2 s) periods of silence in the call counted within 2 min] before disturbance (Kruskal-Wallis $\chi^2_2=0.194$, $P=0.918$). Therefore the differences among populations in latencies to call were unlikely a result of pre-existing differences in call fragmentation in the absence of disturbance. Latencies to resume calling were not normally distributed and differed significantly among populations ($J^*=4.86$, $P<0.0001$; Fig. 2). Consistent with our prediction, median latencies were longest for Kauai males ($n=30$, median= 20.33 s), intermediate for Hilo males ($n=39$, median= 10.35 s), and shortest for Oahu males ($n=34$, median= 2.74 s).

A chi-square goodness of fit test on the number of times, out of three, an individual resumed calling within the 600 s allotted also corroborated this pattern (χ^2_6 =17.161, $P<0.0087$; Fig. 3). Although some of the cells in the contingency table had expected values of less than 5, we followed Rosenthal and Rosnow's (1991) suggestion that such a criterion is unnecessarily strict when n is large ($n=88$, in this case). In any case, when we collapsed cells to correct for this issue such that the number of individuals that resumed calling all three times and the number of individuals that never resumed calling were
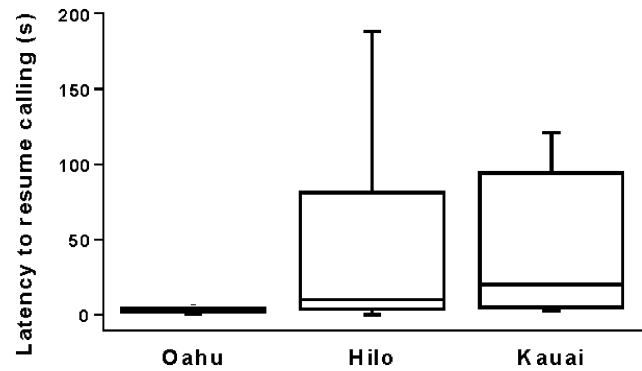

Fig. 2 Box plot of latencies to resume calling following a disturbance in male *T. oceanicus* from Oahu ($n=34$), Hilo ($n=39$), and Kauai ($n=30$). *Boxes* enclose 50% of the observations, and *lines within boxes* represent median values. *Bars* indicate 85% quantiles. Although males were measured more than once, only the shortest latency time (*s*) for each male was used to create this box plot
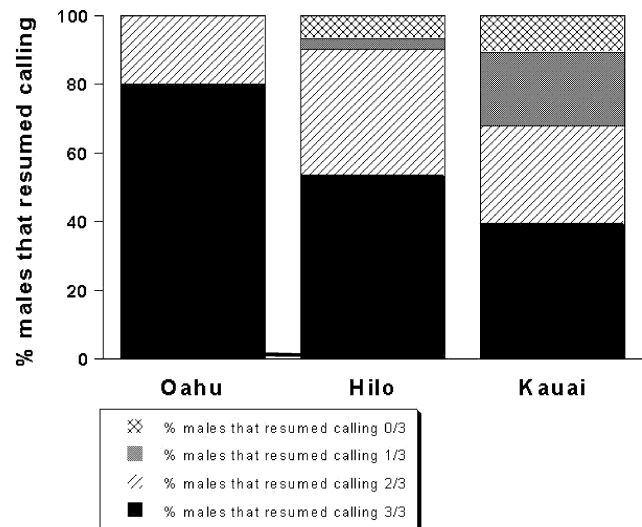

Fig. 3 Comparison of the percentage of *T. oceanicus* males from Oahu ($n=30$), Hilo ($n=30$), and Kauai ($n=28$) that resumed calling 0, 1, 2, or 3 times in the allotted time (600 s) out of three disturbance trials (χ^2_6 =20.351, $P<0.0024$)

compared among populations, the same population pattern remained (χ^2_2 =10.757, $P<0.0046$).

Discussion

The latencies to resume calling after a disturbance among the three Hawaiian populations followed the expected pattern. Males from Oahu, where the risk of parasitism is lowest, had the significantly shortest latencies to call (Fig. 2), and were most likely to resume calling within the allotted period of time after being disturbed (Fig. 3). Males from Hilo, where the risk of parasitism is intermediate, had intermediate latencies to call (Fig. 2). Unlike Oahu males, which always resumed calling at least

twice out of three trials, 10% of Hilo males either never resumed calling or only resumed once out of three times (Fig. 2). Males from Kauai, where parasitism risk is the greatest, had the longest latencies to call. Over 30% of Kauai males either never resumed calling or only called once out of three trials (Fig. 3). Because the crickets were all from laboratory populations subject to the same standardized disturbance, the difference among populations appears to be a trait under genetic control, rather than being a function of the environment in which the animals find themselves.

Other acoustically signaling animals have been shown to use silencing as an effective defense against predators (Spangler 1984; Jennions and Backwell 1992). The lesser wax moth, *Achroia grisella*, and the bush katydid, *Insara covilleae*, both use acoustic calls to attract mates. Spangler (1984) demonstrated that both of these insects momentarily ceased calling when they heard either real or pre-recorded predatory bat vocalizations. However, such silencing behavior may not always be effective in avoiding predation or parasitism. *O. ochracea* in Florida that home in on male *Gryllus rubens* calling songs often remain near the source of the call for more than 5 min after the call has ceased, and sometimes remain for over 20 min (Walker 1993). This may represent a behavioral counter-adaptation by the flies. If female *O. ochracea* employ such prolonged waiting behavior in the three Hawaiian islands discussed in this paper, then males with short or even intermediate latencies to call may have little chance at evading these parasitoid flies. However, it is unlikely that a female fly would continue to wait near a silenced cricket if another nearby male cricket was calling. Thus, the effectiveness of using silence as an avoidance mechanism may be dependent upon the proximity of other calling males. Other researchers have also expressed doubt about the effectiveness of silencing behavior for orthopteran hosts of *O. ochracea*. Müller and Robert (2002) demonstrated that even though call fragmentation in *G. rubens* reduces female *O. ochracea* accuracy at localizing the sound source, this reduction in accuracy is quite small (a difference of 5–6 cm). However, the periods of silence the authors inserted into a calling song to create a "highly" fragmented call were quite small and were intended to represent natural variation in an undisturbed call, not a call that has been fragmented due to a disturbance. Thus, the amount of fragmentation observed in our study could have a much larger effect in fly landing accuracy.

Although cessation of calling for certain periods of time may not always prove an effective means of avoiding parasitism (Bullock 1984), it is one of several lines of defense that a male cricket can use to decrease its chances of infection. Periods of silence may make it more difficult for flies to accurately locate their hosts (Müller and Robert 2001), and because of this, a nearby unsilenced male may become a more desirable target. During periods of silence, male crickets are also better able to listen and focus on any other external cues in their environment that could predict an approaching natural enemy (Faure and Hoy 2000). Males in this study were confined to relatively small containers, thus limiting their movement, and were visually isolated from the observer; however the observer was able to audibly detect instances when males jumped after disturbance, and this activity was not uncommon (personal observation). In the field, cessation of calling in response to air disturbances may frequently be coupled with a jumping or running response, which has been demonstrated in another Gryllid (Gras and Hörner 1992). Together, silencing and locomotion may prove a very effective escape response, especially since *O. ochracea* respond to call cessation by maintaining their original flight path (Müller and Robert 2001).

Male *T. oceanicus* face a trade-off between survival and reproduction. Here, we have demonstrated that males from populations with differing degrees of parasitism differ in the degree of caution they exhibit after a disturbance, in terms of their latencies to resume calling. Males from highly parasitized islands take longer to resume calling after a disturbance than males from less parasitized islands. While males with long periods of silence following a disturbance may reduce their chances of parasitism, they are also likely to suffer a decrease in reproductive success if they are less likely to attract females (Hedrick 1986; Wagner 1996).

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