FECUNDITY OF *LARRA BICOLOR* (HYMENOPTERA: CRABRONIDAE) AND ITS IMPLICATIONS IN PARASITOID: HOST INTERACTION WITH MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE: *SCAPTERISCUS*)

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ABSTRACT

Larra bicolor F. (Hymenoptera: Crabronidae) is a specialist parasitoid of *Scapteriscus* (Orthoptera: Gryllotalpidae) mole crickets, attacking adults and medium to large nymphs. Reproductive systems were dissected from 10 female wasps collected in northern Florida. Each had 2 ovaries, each with 3 ovarioles. The maximal number of mature eggs (10) plus developing oocytes (83) was 93. Female wasps deposit an egg on the venter of the host's thorax, and the wasp larva develops as an ectoparasitoid. Twenty newly-emerged female wasps housed in small cages with at least 1 male and with 7 potential hosts replaced daily deposited a mean 2.44 eggs (range 0-10) per day for a total lifetime production averaging 56 eggs (range 17-91) during a lifespan averaging 23.5 d (range 8-40). Assuming 3 wasp generations with fecundity as shown to 1 host generation, per year, the wasp should easily be able to outreproduce its host mole crickets. A few of the hosts became superparasitized with 2 or even 3 eggs, but at most 1 larva of *L. bicolor* developed successfully on each host, so superparasitism is a disadvantage; its incidence in the laboratory (<2%) and field (3%) was low.

Key Words: reproduction, ovarian structure, superparasitism, fertility

RESUMEN

La avispa *Larra bicolor* F. (Hymenoptera: Crabronidae) es un parasitoide especializado en grillotopos del género *Scapteriscus* (Orthoptera: Gryllotalpidae) que ataca a adultos y ninfas de tamaños medio y grande. Se diseccionaron los sistemas reproductivos de 10 hembras colectadas en el norte de Florida. Cada uno tenía dos ovarios con tres ovarioles. El número máximo de huevos maduros (10), mas los oocitos en desarrollo (83), fue 93. Las hembras depositan un huevo sobre la superficie ventral del tórax del hospedero, y la larva de la avispa se desarrolla externamente como ectoparásito. Viente hembras recientemente eclosionadas y mantenidas en jaulas pequeñas, junto con por lo menos un macho y siete hospederos potenciales reemplazados diariamente, ovipositaron un promedio de 2.44 huevos (rango 0-10) por día para una producción total de un promedio de 56 huevos (rango 17-91) durante una vida de promedio de 23.5 días (rango 8-40). En tres generaciones de la avispa, con la fecundidad potencial demostrada y contra una sola generación de grillotopos, esta avispa podría producir más descendencia que su hospedero. Pocos de los hospederos fueron superparasitizados con dos, o raramente tres, huevos. Al máximo solamente una larva de *L. bicolor* se desarrolló exitosamente en cada grillotopo, así que el superparasitismo es un detrimento para la avispa. La incidencia de superparasitismo en el laboratorio (<2%) y el campo (3%) fue baja.

Translation provided by the authors.

Larra spp. have traditionally been called wasps although they are bee-relatives (Hymenoptera: Apoidea). Formerly classified in Sphecidae, they are now considered to belong to Crabronidae in accord with online information by W. J. Pulawski (O'Neill 2008). All are parasitoids of mole crickets (Orthoptera: Gryllotalpidae) (Menke 1992). *Larra* females differ from typical crabronids in that the paralysis they inflict on their hosts is temporary, and larvae develop externally on active hosts (Steiner 1984). *Larra bicolor* F. is a widely-distributed South American species using various species of *Scapteriscus* mole crickets as hosts (Menke 1992). Stock from Amazonian Brazil (via Puerto Rico) was introduced in 1979 into southern Florida, and stock from Bolivia was introduced in 1988 into northern Florida (Frank et al. 1995) to suppress populations of invasive *Scapteriscus* spp. Adults of the 2 stocks can be distinguished by density of punctuation of the head (Menke 1992). By 2005, progeny of the Bolivian stock was widely distributed whereas the Brazilian stock occupied a very restricted area in Broward County (Frank & Walker 2006). Sup-

pression is occurring, but its level is not clear because the wasp has several overlapping annual generations although the hosts have only 1, at least in northern Florida (Frank & Walker 2006). Furthermore, only the adults and mid-sized and larger nymphs of the hosts are susceptible, with invulnerable small nymphs present in summer, and the wasp is inactive underground in the pupal stage during winter (Cabrera-Mireles 2002).

Castner (1988) described the diurnal hunting behavior by *L. bicolor* females (Brazilian stock), and noted that 2 to 3 eggs per female per day were usually produced under laboratory conditions. Castner (1986) observed superparasitism under artificial conditions (glass test tubes). Much more information is necessary for calculating population effects of the parasitoid on the host, which is 1 of our long-term objectives.

Using wasps of the Bolivian stock from Alachua County, Florida, we determined the structure of the wasp's ovaries and ovarioles in order to assess potential fecundity. We report the lifetime egg production by caged females to examine actual fecundity. We observed the outcome of superparasitism and compared its magnitude in cages and in the field.

Materials and Methods

Ovarian Structure

In Jun and Jul 2007, 10 female *L. bicolor* were collected at a field site with nectar-source plants at 29°45' N, 82°17' W, north of Gainesville, Alachua County, Florida. These wasps were transported to the laboratory and chilled in a refrigerator to anesthetize them. The last abdominal segment was grasped with forceps and pulled away from the anterior segments bringing with it the reproductive tract. The reproductive organs were then placed in a small Petri dish filled with ice-cold phosphate-buffered saline solution (PBS, pH 7.0). Ovaries were dissected and rinsed in fresh PBS, and equilibrated overnight in small vials with 30% glycerol in PBS. They were then moved to neutral-red staining solution in watch glasses. After 2 min, they were removed from the stain and washed for 5 min in a 5-mL beaker of ice-cold PBS. The common oviduct was removed, and right and left ovaries were separated and temporarily mounted on a slide in 30% glycerol/PBS. The ovaries were photographed with a stereomicroscope (Leica Microsystems, Wetzlar, Germany) fitted with an Auto-Montage digital photography system (Syncroscopy, Frederick, MD). Images were taken at 10× magnification and were enhanced with Photoshop 5.5 graphics-editing software (Adobe Systems, San Jose, CA). Auto-Montage image-processing software was used to scale the images. Means, standard errors, and ranges were calculated for ovariole length, mature egg number, mature egg length, oocyte number, and largest and smallest oocyte length. Correlation coefficients were computed with SAS 9.1 (SAS Institute, Cary, NC).

Lifetime Egg Production

Larvae of *L. bicolor* collected on mole crickets at a field site at 29°50' N, 82°04' W in Bradford County, Florida were brought to the laboratory in autumn 2006 and allowed to pupate in 92-mL (25 dram) transparent styrene vials with moist clean sand. The newly-emerged adult wasps were used in this experiment in May-Jun 2007. Females were weighed as soon as they were observed to have emerged at daily check, and then placed into $30 \times 30 \times 30$-cm cages, 1 wasp per cage, in a greenhouse with maximum temperature regulated not to exceed 30°C. One or more newly-emerged male *L. bicolor* were added to try to ensure mating. Into each cage was placed a flowering *Spermacoce verticillata* L. (Rubiaceae), a favored nectar source, in a 4-L pot together with an artificial nectary (a small glass vial fitted with a cotton wick and containing a mix of honey and 20% sucrose solution). Wasps were noted to visit flowers and artificial nectaries. Moist sand was placed on the floor of each cage to a depth of 2.5 cm, and it was kept moist by spraying twice daily with tap water.

Seven parasitoid-free, healthy adult *Scapteriscus* mole crickets were added to each cage. The number 7 was a compromise based on Castner's (1988) observation that 2-3 eggs were laid per female per day, consistent with (a) our intent that the number of available hosts should exceed the number of eggs likely to be laid, and (b) with the number of mole crickets we had in stock. Even so, it called for 140 fresh mole crickets daily over a period of weeks. All 3 species occurring in Florida are hosts for the Brazilian stock *L. bicolor* (Castner 1984). We used a mixture of 4 or 5 *S. vicinus* Scudder, field-collected at sites near Gainesville and laboratory-maintained, and 3 or 2 *S. abbreviatus* Scudder, from a long-maintained laboratory culture originating from specimens collected in southeastern Florida. These mole crickets were left in the cage for ≈24 h. All mole crickets were removed from each cage after sunset, when *L. bicolor* activity had ceased, and examined for the presence of *L. bicolor* eggs. By the use of sharp-pointed forceps, every egg detected on the surface of a mole cricket was removed from its host and recorded. Seven mole crickets were placed back into each cage, but mole crickets from which eggs had been removed were not recycled into cages until ≥3 d had passed. This procedure was followed daily with the mole crickets until the female wasp (whose presence was checked daily) had died. The routine was followed until data were collected for the progeny of 20 female wasps. Means and standard errors were computed for

the variables of wasp weight, lifespan (days), eggs laid per day, and total number of eggs produced. Correlation coefficients were calculated between paired variables with SAS 9.1 (SAS Institute, Cary, NC).

Superparasitism

Mole crickets possessing 2 or more wasp eggs were detected during the lifetime egg production experiment. The number and general location of eggs were recorded. These mole crickets were placed individually into 92-mL (25 dram) transparent styrene vials with moist clean sand. Vials were housed in an environmental chamber (Walker et al. 1993) at 27°C, 55% RH and 16:8 h L:D. Mole crickets were fed "FRM Cricket and Worm Feed" (Flint River Mill, Bainbridge, GA) at least twice per week, and water was added to the substrate as needed. Mole cricket condition and parasitoid development were monitored by visual inspection through the transparent wall of the vial, or by removing the mole cricket only when the wasp larva could not be seen through the vial wall.

Mole crickets were trapped in pitfalls in a pasture in Bradford County (locality given above), in Oct-Dec 2005 and Aug-Dec 2006 (Portman 2007) in a field project to be reported elsewhere. They were brought to the laboratory for examination, and the presence of *L. bicolor* eggs and larvae was recorded. This provided a record of superparasitism in the field.

Results

Ovarian Structure

All wasps dissected had 2 ovaries, each with 3 ovarioles (Fig. 1) typical of crabronids (Ohl & Linde 2003). There were 7.60 ± 0.63 (mean ± SE, range 4-10) mature eggs and 69.0 ± 3.30 (range 54-83) developing oocytes. Egg length was 1.63 ± 0.03 mm (range 1.50-1.80). Ovariole length correlated with egg load (n = 10, r = 0.81, P < 0.004). Egg load correlated negatively with average egg length (n = 10, r = -0.70, P < 0.03). The maximal number of mature eggs (10) plus developing oocytes (83) was 93, albeit in different individuals.

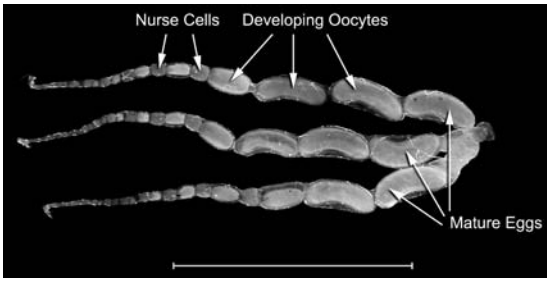

Fig. 1. Ovary of *L. bicolor* stained with neutral red and mounted in 30% glycerol. Scale line = 5.0 mm.

Lifetime Egg Production

All wasps oviposited, but we could not distinguish male eggs from female eggs, and we do not know whether every female mated. The few matings observed were very brief (seconds). The number of eggs laid may be underestimated if the ovipositing females removed any existing eggs before depositing a new one. Castner (1986) observed removal of eggs under the unusual circumstance that he provided only 1 potential mole cricket host per ovipositing wasp. However, (a) the maximal number of mature eggs deposited during 1 d was 10, which matches the maximal number of mature eggs found by dissection, and (b) the maximal lifetime egg output was 91, which is very close to the maximal number of mature eggs plus developing oocytes (93) found by dissection (Table 1). Thus, the 2 evaluations by differing methods support each other. The rate of oviposition correlated with the initial weight of female wasps (n = 20, r = 0.68, P = 0.006), and the total egg production correlated with lifespan (n = 20, r = 0.80, P = 0.000).

Superparasitism

Twenty mole crickets were superparasitized, having 2 or 3 sibling eggs (<2% of the observations). Ten of the 20 had 2 eggs attached on opposite sides (L-R) of the venter of the thorax. Nine had 2 eggs attached adjacently. One had 3 eggs, 2 adjacent and 1 opposite (Fig. 2). After hatching, every larva attached without relocating and without attacking a sibling. In no instance did more than 1 larva per host survive to pupation, and in 10 instances (5 with the 2 eggs adjacent, and 5 with 2 eggs opposite) both larvae died. Survival to pupation was only 24.4% overall, which contrasts with survival of 97.6% reported for single *L. bicolor* larvae (Cabrera-Mireles 2002).

Discussion

Although superparasitism occurred, it was a disadvantage. The experimental method did not allow observation of females ovipositing, and it is unknown whether females removed any of the eggs they found. The strategy followed by most of the female wasps (of the Brazilian stock) observed by Castner (1986), of removing an existing egg before depositing a new one, would have allowed the new egg a much greater chance of developing. However, those females may not have been able, after 24 h, to distinguish their own eggs from those of other females. The simplest hypothesis with the new observations is that female wasps

TABLE 1. LIFE HISTORY TRAITS OF *L. BICOLOR* FEMALES. FOR EACH MEASUREMENT $N = 20$.

Trait	Mean ± SEM	Minimum	Maximum
Wasp weight (mg)	107.43 ± 8.71	58	185
Lifespan (d)	23.5 ± 1.87	8	40
Lifetime egg output	56.05 ± 4.38	17	91
Daily egg output	2.44 ± 0.14	0	10

could recognize their own previously-deposited eggs and chose to deposit another without removing the earlier one(s). If a female's normal behavior is to move away from the proximity of a host she has parasitized, the chance of re-encounter with a host that she had parasitized might be reduced. Overall, superparasitized mole crickets amounted to <2% of the laboratory observations. However, the incidence of superparasitism in mole crickets collected from pitfall traps in the field was 3% (9 out of 304 parasitized mole crickets had 2 wasp eggs whereas all others had a single egg or single larva), so the cage experiments were a reasonable approximation of events in the field.

The new data on lifespan, and on daily and lifetime egg output place us a few steps closer to understanding the dynamics of *L. bicolor* populations in northern Florida. At 26 ± 2°C and 65 ± 5% RH, *L. bicolor* (Brazilian stock) eggs incubate for 6-7 d, the 5 instars develop in 10-11 d at the end of which the host dies, a further day is spent constructing a cocoon, and the pupal duration is 6-8 weeks (Castner 1988). In an aseasonal tropical climate with continuous host availability, this might give the capability of ≈4.5 generations each year, but because of variability among individuals in development time and because each female oviposits through her adult lifespan, we would expect completely overlapping generations. Therefore, it would not be possible to distinguish generations in the field. Effects of insect parasitoid populations on host populations are almost always calculated based on 1 discrete generation of parasitoid per host generation, so such a calculation would not be possible.

Fig. 2. Venter of the thorax of a *Scapteriscus* mole cricket bearing 3 eggs deposited by 1 *L. bicolor* female within 1 d. All are between the 1st and 2nd pairs of legs and each is ≈1.6 mm long.

Northern Florida has a seasonal climate with freezing temperatures in winter, and *L. bicolor* adults are killed by hard frost (Cabrera-Mireles 2002). The duration of the pupal stage is highly variable with range 45-238 d; at least some pupae late in the autumn enter diapause, which is not broken until the following spring, with adults emerging throughout May in outdoor, underground rearings (Cabrera-Mireles 2002). Overwintered pupae reared from field-collected larvae late in autumn 2006 and placed in an environmental chamber (Walker et al. 1993) at 27°, 55% RH and 16:8 hr L:D, produced adults the following Apr and May (Portman, unpublished data). The prolonged emergence period further contributes to the overlapping generations and lack of distinct cohorts. Adults have been observed as early as late Apr and as late (in the mild winters of 2006-2007 and 2007-2008) as mid-Jan (Frank, unpublished data). If 4 months of the year (typically mid-Dec to mid-Apr) are the period during which *L. bicolor* adults are inactive, then the number of potential generations must be reduced from ≈4.5 (as they might be in the tropics) by a third, to perhaps 3 (or even 3.5 if the first hard frost does not arrive until mid-Jan).

The life cycle of *Scapteriscus* mole crickets, too, is influenced by climate. The 2 species in northern Florida have only 1 generation each year. They oviposit in spring, after which overwintered adults die. Young nymphs are not suitable hosts for *L. bicolor*. These dynamics affect host availability for *L. bicolor*.

If the lifetime egg output of each *L. bicolor* female averages 56, and if there were 3 parasitoid generations during each annual generation of the host, and if the host's lifetime egg output averages 90 eggs (an approximation for 3 clutches spaced 3 weeks apart, each of 30 eggs), the calculation of reproductive potential for parasitoid and host would be simple. The parasitoid would be able to out-reproduce the host by far.

At least by Aug there is no evidence of discrete generations among adult wasps in the field. Standardized semi-weekly counts of adult *L. bicolor* feeding in Aug-Nov 2006 on nectar of *Spermacoce verticillata* in 3 plots at each of 2 sites near Gainesville, Florida revealed average monthly counts from Aug to Nov ranging from 25.7 to 40.7, but only 17.2 in Jul (Portman 2007). Wasp activity was not monitored after Nov, although it probably continued until the first hard frost in mid-Jan 2007. The plants used were transplanted from a greenhouse into the field in Mar 2006. Routine monitoring of the plants for wasps began in May, but wasps were not observed until Jul. The average monthly count of only 17.2 wasps in Jul could have resulted from the small size of the plants at that time (so they were less attractive than later) or from relatively few wasps present during that month. Host availability is very low in Jul due to its life cycle; few overwintered adults remain alive and nymphs are small (Walker 1985). The overlapping of generations in *L. bicolor* is promoted by (1) the spread in time of emergence of adult wasps from pupae, and (2) the spread in time due to the long ovipositional period (≥ 3 weeks) of each female wasp. As a result, mole crickets are vulnerable to daily attack by the wasps at least throughout the autumn until the first hard frost.

Calculation of mortality caused by the wasp during the warmer months more closely resembles a study in epidemiology than it resembles a textbook calculation of parasitoid: host relationships. Special considerations for this situation are that the level of daily attack apparently depends upon (1) the number of eggs that each *L. bicolor* female can lay per day (mean = 2.44), and (2) the ratio of female *L. bicolor* to susceptible mole crickets in an area at a given time. Any host found parasitized by an *L. bicolor* egg on d x would have died and thus been removed from the population not later than d x + 18 (maximum time for egg and larval development). Any host found parasitized by an *L. bicolor* larva on d x would have died and thus been removed from the population not later than d x + 11 (maximum time for all instars). The percentage parasitism of the host by wasp eggs or larvae is the measurable quantity in the field that can be used to calculate generational mortality of the host. Further research is necessary to deal with such complex models.

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