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REPRODUCTIVE INCOMPATIBILITY BETWEEN TWO SUBSPECIES OF *COLEOMEGILLA MACULATA* (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

There is interest in introducing the midwestern subspecies of *Coleomegilla maculata* (DeGeer), *C. m. lengi* Timberlake, as a biological control agent for augmentation programs in Florida. The Division of Plant Industry (DPI) of the Florida Department of Agriculture and Consumer Services has prohibited the release of *C. m. lengi* for fear that it could interbreed with the native Florida subspecies *C. m. fuscilabris* (Mulsant), causing genetic contamination or suppression of the C. *m. fuscilabris* populations. Two populations of these subspecies, *fuscilabris* from the southeastern USA and *lengi* from the midwestern USA, were crossed. Reciprocal single pair crosses were performed under controlled laboratory conditions (temperature, relative humidity and daylength) on two dates. Results demonstrated a nearly complete reproductive incompatibility between these two populations in the first generation (F1) and complete reproductive incompatibility in the second (F2). Further analysis is required to establish the cause of the reproductive incompatibility.

Key Words: *Coleomegilla maculata fuscilabris*, *C. m. lengi*, augmentation, biological control, reciprocal crosses

RESUMEN

Existe interés en introducir la subespecie centro occidental de *Coleomegilla maculata* (DeGeer), *C. m. lengi* Timberlake, como agente controlador en programas de control biológico aumentativo y de conservación en Florida. La División de Industria de las Plantas (DPI) del Departamento de Agricultura y Servicios de Consumo de Florida, prohibió la liberación de esta subespecie por temor que *C. m. lengi* pudiera cruzarse con la subespecie nativa de Florida *C. m. fuscilabris* (Mulsant), causando contaminación genética o supresión de su población. Dos poblaciones de estas dos subespecies de *C. maculata*, *fuscilabris* del sureste de los Estados Unidos y *lengi* de la parte centro occidental, fueron cruzadas. Cruces recíprocos con parejas individuales se llevaron a cabo bajo condiciones controladas de laboratorio (temperatura, humedad relativa y fotoperiodo) en dos fechas diferentes. Los resultados demostraron una incompatibilidad reproductiva casi completa entre estas dos poblaciones durante la primera generación (F1) y una incompatibilidad reproductiva total en la segunda generación (F2). Se requiere mayor investigación y análisis para establecer la causa de dicha incompatibilidad reproductiva.

Coccinellids are among the best-known beneficial insects, with about 500 species found in the United States and Canada (Weeden et al. 1999, White & Darmo 1999). Within this family, the American genus *Coleomegilla* ranges from southern Canada to Venezuela and Perú (Hazzard et al. 1991, Hilbeck & Kennedy 1996).

Coleomegilla maculata (DeGeer) is a new world species widely distributed in North, Central and South America (Gordon 1985, Munyaneza & Obrycki 1998). According to Gordon (1985), there are three subspecies of *C. maculata* in the United States separated on both morphological (spot pattern, color, body size and genitalia) and geographical criteria. In the United States there is a southeastern species, *C. m. fuscilabris* (Mulsant), a midwestern subspecies, *C. m. lengi* Timberlake, and a western subspecies, *C. m. strenua* (Casey) (Gordon 1985). No breeding studies had been conducted between these populations (Krafsur et al. 1995), until recently. Krafsur & Obrycki (2000), performed reciprocal crosses between and within populations of *C. m. lengi* from Iowa, *C. m. strenua* from Texas, and a Honduran population of *C. m. medialis*.

Differences also have been observed in temperature and humidity preferences among the subspecies (J. White & K. Gallagher, Entomos LLC., Gainesville, Florida, pers. comm.).

C. maculata is considered an efficient predator and is known to prey on eggs and larvae of many economically important coleopteran and lepidopteran pests (Andow & Risch 1985, Coll & Bottrell 1991, Hazzard & Ferro 1991, Giroux et al. 1995, Acosta 1998, Cottrell & Yeargan 1998a, Vigneault et al. 1998), on several aphid pest species (Andow & Risch 1985, Groden et al. 1990, Giles et al. 1994, Acosta 1998, Harmon et al. 1998, Obrycki et al. 1998a, 1998b, Phoofolo & Obrycki 1998), and on other food sources such as fungal spores and plant pollen (Smith 1961, Munyaneza & Obrycki 1997, Cottrell & Yeargan 1998b). Based on the voracity and efficiency of this predator toward economically important pests, *C. maculata* is considered an important biological control agent and is used in augmentative and conservation bio-

logical control programs (Cottrell & Yeargan 1999, Nault & Kennedy 2000, J. White, Entomos LLC., Gainesville, FL, pers. comm.).

In Florida, the native subspecies *C. m. fuscilabris* is successfully used in augmentation biological control programs as a broad-spectrum biological control agent against insect and mite pests in nurseries and greenhouses (J. White, Entomos LLC., Gainesville, FL, pers. comm.). The midwestern subspecies *C. m. lengi* is being used in nurseries and greenhouses in North and South Carolina for the control of insect and mite pests (J. White, Entomos LLC., Gainesville, FL, pers. comm.).

There is interest in introducing *C. m. lengi* into Florida but the Division of Plant Industry (DPI) of the Florida Department of Agriculture and Consumer Services (FDACS) has prohibited release of *C. m. lengi* in Florida. The DPI is concerned that the two subspecies could interbreed, leading to genetic contamination or even elimination of the Florida subspecies *C. m. fuscilabris* (J. White, Entomos LLC., Gainesville, FL, pers. comm.).

The objective of this study is to evaluate whether two populations of *C. maculata, C. m. fuscilabris* from Florida and *C. m. lengi* from North Carolina and Louisiana, will interbreed and produce viable progeny under laboratory conditions.

Materials and Methods

Colony Source

Colonies of *C. m. fuscilabris* and *C. m. lengi* were obtained from the laboratories of Entomos LLC., in Gainesville, Florida. The *fuscilabris* colonies were collected from Florida, and the *lengi* colonies were originally obtained from North Carolina and northern Louisiana (J. White, Entomos LLC., Gainesville, FL, pers. comm.).

Rearing Methods

In total, 50 adults of both sexes of *C. m. fuscilabris* and 50 of *C. m. lengi* were used to initiate colonies reared in the Department of Entomology and Nematology at the University of Florida, Gainesville.

Each colony was reared in plastic containers ($31.5 \times 24 \times 10$ cm) under quarantine conditions with a controlled temperature of 24° to 26°C, a relative humidity of 60 to 63%, and a photoperiod of 16 h of light and 8 h of darkness (16L:8D). All containers contained 4 waterers, 2 made of 30 ml plastic cups with snap-on lids (P100: PL1, Solo Corporation, Chicago, IL), and 2 of 60 ml plastic cups with snap-on lids (B200: PL2, Solo Corporation, Chicago, IL). In the center of each lid a hole was made through which 10 cm of dental wick was introduced into the plastic cup, allowing 1 cm of the wick to serve as a water source for the coccinellids. All water containers were filled with distilled water every other day.

Food was provided every other day and consisted of approximately 1.5 g of frozen bee pollen (Sigma Inc., St. Louis, MO) placed on 2 plastic lids (PL1), approximately 2 g of frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Beneficial Insectary, Oak Run, CA) which were sprinkled throughout each container, and 6 ml of a gelatin form of artificial diet provided by Entomos LLC. (Gainesville, FL) on plastic lids (PL2).

Adults and larvae of *Coleomegilla maculata* are known to cannibalize eggs and larvae (Cottrell & Yeargan 1998a, 1998b). To reduce cannibalism, shredded wax paper was added to the rearing containers to isolate individuals. All containers were cleaned every other day to reduce fungal growth on diet and feces.

Four oviposition substrates were put into each colony each day. The oviposition substrate consisted of layers of cotton cut in rectangles (5×2 cm). Each day, egg clusters were cut off the cotton and placed into nursery containers ($1 \times 7 \times 4$ cm) with 3 pieces of sponge ($3 \times 1 \times 1$ cm) that were watered every day to maintain a high level of humidity. All containers were carefully labeled and kept separate to maintain pure colonies. After the eggs hatched, first instar larvae were transferred to bigger containers ($14 \times 14 \times 4$ cm or $22 \times 13 \times 7$ cm) and watered, fed, and cleaned every other day until they reached the pupal stage.

Pupae were removed from the larval containers and individually isolated in small plastic containers ($5 \times 5 \times 2$ cm) with a small wet sponge that was watered every day until adults emerged.

This process was repeated until it was possible to obtain 240 virgin adults (72 females and 48 males of *C. m. fuscilabris* and 72 females and 48 males of *C. m. lengi*).

Compatibility Tests

In total 80 single pair crosses were set up (40 each on two dates): 10 female *fuscilabris* × male *fuscilabris* ($f \times f$), 10 female *fuscilabris* × male *lengi* ($f \times l$), 10 female *lengi* × male *lengi* ($l \times l$), 10 female *lengi* × male *fuscilabris* ($l \times f$). In addition, 10 virgin *fuscilabris* females and 10 virgin *lengi* females were held individually as controls to determine if virgin females can deposit viable eggs.

Each pair was placed into a plastic container ($10 \times 7 \times 4$ cm) with wax paper substrate, oviposition substrate, one 7.5 ml water receptacle with cotton wick, *Ephestia* eggs, bee pollen, and 1 ml of artificial diet. The containers were held at 24° to 26°C and a relative humidity of 60 to 63% under a photoperiod of 16L:8D. Every other day all pairs were fed, watered and the containers cleaned.

The pairs were allowed to mate for 4 days and the few eggs laid were discarded during this period because most do not hatch (White & Darmo 1999). Over the next 5 days the number of eggs laid was counted daily and isolated in plastic con-

tainers (5 × 5 × 2 cm), with 1 to 45 eggs per container. Larvae that emerged were moved to larger plastic containers (14 × 14 × 4 and 10 × 7 × 4 cm) in groups of approximately 1 to 33 larvae, to reduce cannibalism.

Egg counts, assessment of viability, and sex ratio were determined with a dissecting microscope. Sexing was based on adult body size (females are bigger), and abdominal morphology (males have a distinct ventral notch in the posterior margin of the posterior abdominal tergite, through which the penis protrudes during copulation, whereas females have a more rounded margin of the posterior tergite) (Hurst et al. 1996). Mating behavior was also observed because the male's intromittent organ can be observed during attempts to copulate and females initially constrict their abdomen to avoid this probing.

The F1 progeny from the reciprocal crosses were reared to adults and allowed to mate *en masse*. The number of F2 eggs laid over 10 days and their viability were recorded.

At the end of the experiment all insects were dissected to confirm their sex and stored in 95% EtOH at -80°C in the Biological Control Laboratory of the Entomology and Nematology Department at the University of Florida.

Results and Discussion

Oviposition

The mean number of eggs laid (±S.D.) in all crosses during 5 days was determined by the subspecies to which the mother belonged (Fig. 1). In the reciprocal crosses (*f* × *l*, *l* × *f*), *fuscilabris* females laid 109.4 ± 11.8 eggs and *lengi* females 86.9 ± 11.7 eggs. In the control crosses (*f* × *f*, *l* × *l*), *fuscilabris* females laid 109.9 ± 17.8 eggs and *lengi* females 88.2 ± 13.2 eggs. The number of eggs laid by the *fuscilabris* females in both reciprocal and control crosses was higher than the number of eggs laid by *lengi* females. This represents a statistically significant difference of 21% in the reciprocal crosses and 19.7% in the controls (paired t = 4.5, α = 0.05, df = 18). This difference between *fuscilabris* and *lengi* females could be due to the fact that the *fuscilabris* subspecies is native to Florida. The temperature (24° to 26°C) and relative humidity (60 to 63%) conditions under which the experiment was conducted were close to the optimal breeding conditions of *C. m. fuscilabris*; this colony of *C. m. lengi* apparently performs better at lower temperatures and relative humidity (J. White & K. Gallagher, Entomos LLC., Gainesville, FL, pers. comm.).

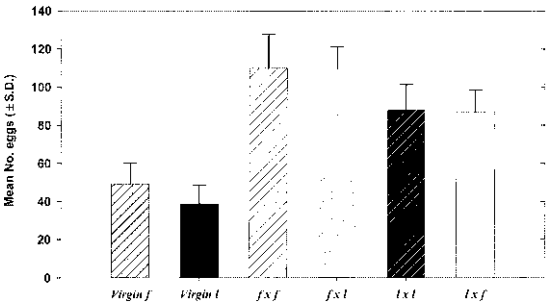

Fig. 1. Mean (±S.D.) number of eggs laid by the controls (virgin *C. m. fuscilabris* (f), virgin C. m. lengi (l), f x f, l x l) and reciprocal crosses (f × l, l × f) during 5 days at 24°-26°C, 60-63% relative humidity and a daylength of 16L:8D.

There was no significant difference (paired t = 0.12, α = 0.05, df = 18) between the mean number of eggs laid by *fuscilabris* females in the reciprocal crosses (109.4 ± 11.8) and in the controls (109.9 ± 17.8) (Fig. 1). The *lengi* females deposited 86.9 ± 11.7 eggs in the reciprocal crosses and 88.2 ± 13.2 eggs in the controls, which is not significantly different (paired t = 0.33, α = 0.05, df = 18) (Fig. 1). The fact that *fuscilabris* females deposited the same number of eggs in the reciprocal and control crosses indicates that the male has no effect on the number of eggs laid by the female. Likewise, *lengi* females deposited the same number of eggs whether they were mated with *lengi* or *fuscilabris* males. However, the presence of a male clearly increases the number of eggs laid by the females.

Virgin females deposited significantly fewer eggs (*fuscilabris*: = 49.3 ± 10.8, paired t = 12.2, α = 0.05, df = 18); *lengi*: = 38.7 ± 10, paired t = 12.7, α = 0.05, df = 18) than mated females (Fig. 1). None of the eggs deposited by the virgin females produced viable larvae.

The crosses between the F1 progeny that developed from the reciprocal crosses (*f* × *l*, *l* × *f*) yielded a total of 7729 F2 eggs (182 eggs from the *f* × *l* crosses and 7547 eggs from the *l* × *f* crosses). The small number of F2 eggs laid by the F1 females from the *f* × *l* cross indicates that a high degree of reproductive isolation exists between these two populations In the second generation. by contrast, more than 7000 F2 eggs were produced by the reciprocal *l* × *f* cross. We have no explanation for the differences in egg production in these F2 generations, but the incompatibility was confirmed when hatchability was examined.

Hatchability

In the female *fuscilabris* × male *lengi* crosses, the egg hatch rate of F1 eggs was low (0.2%), as it was for the reciprocal female *lengi* × male *fuscilabris* crosses (9.1%) (Fig. 2). In the controls (*f* × *f*, *l* × *l*), 78.6% of the *fuscilabris* eggs and 74.4% of the *lengi* eggs hatched. The differences in hatchability of eggs from the reciprocal and control crosses were significant (paired t *fuscilabris* = 43.5, paired t *lengi* = 29.1, α = 0.05, df =18). All eggs deposited by unmated *fuscilabris* and *lengi* females failed to hatch (Fig. 2). These results indicate that

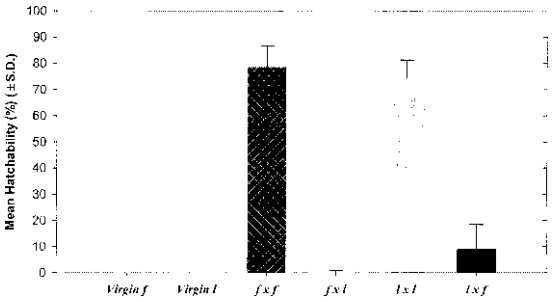

Fig. 2. Mean (±S.D.) hatchability of eggs laid by the controls (virgin *C. m. fuscilabris* (f), virgin C. m. lengi (l), f × f, l × l) and reciprocal crosses (f × l, l × f) during 5 days at 24°-26°C, 60-63% relative humidity and a daylength of 16L:8D.

a high degree of reproductive incompatibility occurred in the reciprocal crosses, when only a few F1 eggs hatched. In the second generation, none of the 7729 F2 eggs laid by the F1 adult progeny hatched. This suggests complete reproductive incompatibility between these two populations by the second generation.

Survival to Adulthood

Survival to adulthood of the progeny produced by the control crosses ($f \times f$ and $l \times l$) was 67.1 ± 7.5 and 59 ± 10.8%, respectively. Survival to adulthood of the 3 F1 eggs that hatched from the female *fuscilabris* × male *lengi* crosses and of the 135 F1 eggs produced by the female *lengi* × male *fuscilabris* crosses was 100 and 7.5 ± 7.9%, respectively. The F1 progeny that resulted from both reciprocal crosses phenotypically resembled the *lengi* parent, having similar spot patterns, color and body size.

The proportion of *fuscilabris* and *lengi* individuals that developed from the egg to the adult stage in the control crosses was comparable to that observed by White & Gallagher (Entomos LLC., Gainesville, FL, pers. comm.).

No backcrosses (F1 × f and F1 × l) were conducted because few F1 individuals survived to adulthood from the eggs laid by one of the reciprocal crosses ($f \times l$ = 3 adults).

Sex Ratio

The sex ratio of the progeny was not different from 1:1 in the control crosses. *C. m. fuscilabris* control crosses produced 49.4% females and 50.6% males. For *C. m. lengi* controls, 50.8% of the progeny were female and 49.2% male. The reciprocal cross of female *lengi* × male *fuscilabris* produced 54.8% female and 45.2% male progeny. The sex ratio of the progeny from the crosses between female *fuscilabris* × male *lengi* was not calculated because only three progeny (2 females and 1 male) were produced.

Since this species is known to live from 3 to 12 months and females can lay from 200 to over 1000 eggs (Acosta 1998, White & Darmo 1999), the 5 day oviposition interval, during which comparative fecundity, subsequent hatchability, successful development to adulthood, and sex ratio of the progeny were analyzed, constituted only a small portion of their total fecundity.

Polymerase chain reaction (PCR) analysis of the *wsp* sequence of *Wolbachia* indicated *Wolbachia* was present in both populations of *C. m. fuscilabris* and *C. m. lengi* (Jeyaprakash & Hoy 2000). The PCR products were sequenced and phylogenetically analyzed, revealing that each population has a different *Wolbachia* strain (Jeyaprakash & Hoy 2000). The 1 to 1 sex ratio observed in both the reciprocal crosses and controls suggests that *Wolbachia* did not have any effect on the sex ratio of these *C. maculata* populations.

The presence of a different *Wolbachia* strain in each population of *C. maculata* could be the basis for the nearly complete reproductive incompatibility in the F1 generation, and a total reproductive incompatibility by the second generation (F2), but further analyses are necessary to confirm this. It also is possible that the incompatibility is due to nuclear genetic differences between the two subspecies.

Krafsur & Obrycki (2000) found the North American populations (*C. m. lengi* and *C. m. strenua*) were intrafertile and interfertile suggesting they are the same species, as Gordon (1985) also considered. The Honduran population (*C. m. medialis*) was only intrafertile. The reciprocal crosses between the American populations (*C. m. lengi* and *C. m. strenua)* and the Honduran population (*C. m. medialis*) were completely sterile suggesting *Coleomegilla maculata* is a species complex, with *C. m. medialis* apparently a different species from *C. m. lengi* and *C. m. strenua* (Krafsur & Obrycki 2000).

Our results are consistent with the possibility that *C. m. lengi* and *C. m. fuscilabris* are also different species. Johnson (1910) considered the possibility that the subspecies *C. m. fuscilabris* [called *C. m. floridiana* by Johnson (1910)] might be a separate species that originated in Cuba.

In conclusion, these data show that if this midwestern population of *C. m. lengi* were released in Florida, viable hybrids are unlikely to be produced with *C. m. fuscilabris*, which would reduce the risk of genetic contamination or suppression of the Florida subspecies. However, tests with larger sample sizes and different populations of *C. m. lengi* and *C. m. fuscilabris,* including backcrosses, are desirable before such releases are permitted.

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