

Relationships Between Adult Abdominal Color and Reproductive Potential in *Diaphorina citri* (Hemiptera: Psyllidae)

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ABSTRACT *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a vector of huanglongbing (citrus greening disease), exhibits three more or less distinct abdominal colors in the adult stage: gray/brown, blue/green, and orange/yellow. A previous report showed that, relative to blue/green individuals, gray/brown individuals of both sexes have lower body mass, and gray/brown females may have lower reproductive output. In the present investigation, we directly examined the relationships between female and male abdominal color and reproductive output. We also assessed how psyllid body mass was influenced by transfer to fresh plant material and how female and male abdominal color affected behavioral responses of males to crushed females in a petri dish assay. Relative to blue/green females, gray/brown females exhibited lower fecundity over the first 2–5 d after mating, but fecundity did not differ over the remainder of a 21-d observation period. Regardless of abdominal color, females mated with gray/brown males showed an earlier, sharper peak in fecundity, whereas females mated with blue/green males showed a later, more gradual peak in fecundity. Gray/brown males elicited lower egg fertility in their mates, but egg fertility did not vary according to female abdominal color. Males—irrespective of abdominal color—exhibited stronger evidence of attraction to crushed blue/green females than to crushed gray/brown females. Gray/brown individuals of both sexes showed an increase in body mass 5–6 d after transfer to a new citrus seedling, suggesting that abdominal color (which is closely related to body mass) may be influenced at least in part by plant quality.

KEY WORDS Asian citrus psyllid, body color, pigment, reproductive output, fecundity

The psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is a pest of worldwide importance that vectors phloem-limited bacteria (*Candidatus Liberibacter* spp.) responsible for huanglongbing (citrus greening disease) (Halbert and Manjunath 2004, Hung et al. 2004, Bové 2006). *D. citri* was first found in Florida in June 1998 (Tsai et al. 2000) and is now established throughout the state's citrus-growing regions (Michaud 2004). Although feeding and/or oviposition may result in direct damage to plant tissue (Michaud 2004), the primary economic importance of the psyllid is transmission of huanglongbing, one of the world's most serious diseases of citrus (Bové 2006). Infected citrus trees produce unmarketable fruit and may ultimately die from the disease (Halbert and Manjunath 2004, Bové 2006). Huanglongbing was first detected in southern Florida in August 2005 (Halbert 2005) and has been confirmed in 32 counties as of August 2008 (UF/IFAS 2008). In Florida, *Candidatus Liberibacter asiaticus* is the only bacterial agent of the

disease and *D. citri* the only competent vector known to occur. *Trioza erytreae* (Del Guercio) is an Old World vector of huanglongbing that is not found in the United States (Bové 2006). Detailed investigations into the biology, behavior, and ecology of *D. citri* may ultimately lead to improved monitoring and management strategies for this pest of worldwide economic importance.

Variation in body color has been described in numerous species within the superfamily Psylloidea, including *Cacopsylla midorae* (Miyatake) (Inoue 2004); *Cacopsylla elegans* Inoue (Inoue 2004); *Cacopsylla chinensis* (Yang & Li) (Yang et al. 2004); *Agonoscena pistaciae* Burckhardt & Lauterer (Mehrejjad and Copland 2005); and the pear psylla, *Cacopsylla pyricola* (Förster) (Wong and Madsen 1967, Krysan and Higbee 1990), as well as in many species in the family Aphididae (Araya et al. 1996; Watt and Hales 1996; Stotzel and Miller 1998, 2001; Nevo and Coll 2001; Toros et al. 2003). This variation is may be associated with different seasonal forms, endosymbionts (Weisgraber et al. 1971, Jenkins et al. 1999), or (in some aphids) genetically distinct morphs (Araya et al. 1996, Langley et al. 2006). Body color for some species may vary over time within individuals (Jenkins et al. 1999, Nevo and Coll 2001). Based on previous work (Husain and Nath 1927, Skelley and Hoy 2004, Wenninger and Hall 2008a), we recognize three more or less discrete

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color classifications in the abdominal color of adult *D. citri*: gray/brown, blue/green, and orange/yellow. Abdominal color in *D. citri* may change over the life of an individual (Wenninger and Hall 2008a) and thus seems not to represent morphotypes that are exclusively determined genetically or seasonal morphotypes as are found in many sternorrhyncan species in more temperate climates.

Many hemipteran and lepidopteran insects exhibit a green or blue/green color due to a mixture of yellow pigment (including carotenoids) and blue bile pigment, which may be present in the epidermal cells and/or the hemolymph of insects (Cromartie 1959, Barbier 1981, Law and Wells 1989, Saito et al. 1998). Although the early presumptions that blue/green coloration in insects derived from accumulation of chlorophyll have not been supported (Wall 1933, Barbier 1981), the biosynthetic pathways of bile pigments have yet to be fully clarified. The biological significance of variation in body pigmentation also is not fully understood. In aphids that show genetically based color variation, body color has been found to be associated with differences in developmental and reproductive rates (Araya et al. 1996), resistance to parasitism (Ankersmit et al. 1981, 1986), and attack rates by natural enemies (Loosey et al. 1997, Libbrecht et al. 2007).

Previously, Wenninger and Hall (2008a) showed that gray/brown individuals of both sexes in *D. citri* have lower body mass relative to blue/green individuals and found suggestive evidence of lower reproductive output in gray/brown females. In the present investigation, we directly examine whether female fecundity and egg fertility are related to female and male abdominal color. We also explore whether psyllid body mass changes after psyllids held individually on a seedling are transferred to fresh plant material. Finally, we investigate whether behavioral responses of males to crushed females in a petri dish assay vary according to male and female abdominal color, which may inform efforts to identify the putative female sex pheromone (Wenninger et al. 2008). The goal of these studies was to clarify further the reproductive biology and ecology of *D. citri* in relation to abdominal color.

Materials and Methods

Rearing and Housing Psyllids. All adult psyllids used in mating experiments and body mass experiments (see below) were collected as fifth-instar nymphs from a laboratory colony reared on *Murraya paniculata* (L.) Jack at the United States Horticultural Research Laboratory (USHRL), as described by Hall et al. (2007). We transferred nymphs individually to 'Duncan' grapefruit (*Citrus paradisi* Macfayden) seedlings (two- to three-leaf stage) caged in plastic vial containers, described in Wenninger and Hall (2007). Briefly, each cage consisted of a 52-mm-tall vial, modified as an open-ended cylinder with a foam plug used to stopper the top opening and two ventilation holes on the sides; individual cages were slipped over a seedling grown in a cone-shaped planting con-

tainer. Psyllids were held in an environmental chamber at 26°C, 60% RH, and photoperiod of 14:10 (L:D) h, which resulted in 70–80% RH inside the vials. Light intensity (from fluorescent lights) just above the plastic vial containers was $\approx 3,200$ lux.

Psyllids used in behavioral assays (see below) were obtained from a laboratory colony at the University of Florida Citrus Research and Education Center (CREC) maintained on sour orange (*Citrus aurantium* L.) and 'Hamlin' orange (*C. sinensis* [L.] Osbeck), as described by Wenninger et al. (2008). Fifth-instar nymphs were collected from this culture and placed in groups of 40–50 on seedlings uninfested previously by psyllids. These plants were isolated from the parental culture within individual plastic cylinders with fine-mesh screen lids. The nymphs were checked daily to determine the day of adult eclosion, and emerging adult psyllids were segregated by sex and transferred individually to flushing Hamlin seedlings. These plants were isolated as described above. To obtain mated psyllids, pairs of sexually mature adult (3–5 d old) males and females were established and maintained on plants for 24–48 h before initiation of behavioral assays (Wenninger et al. 2008).

Mating Experiment. Adult psyllids were examined daily over the 2–3 d preceding the establishment of female/male pairs and assigned to one of two color categories—gray/brown or blue/green—based on the color of the ventral side of the abdomen (see Wenninger and Hall 2008a). Orange/yellow abdominal color, which is associated with the production of eggs and (in older males) the visibility of internal reproductive organs (Wenninger and Hall 2008a), is not considered in the current investigation. Changes in abdominal color of females over the course of the experiment were not examined because females typically take on an orange/yellow coloration after mating (Wenninger and Hall 2008a), which obscures any underlying blue/green or gray/brown coloration. To initiate mating treatments, males were gently coaxed onto the bristles of a small paintbrush and transferred to a vial that housed a female. Mating pairs were established just before the onset of photophase, and males were removed after 48 h. All psyllids used in experiments were 4–8 d posteclosion at the establishment of treatments; by 4 d of age, male and female *D. citri* are reproductively mature, and females typically begin laying eggs on the same day they are mated (Wenninger and Hall 2007). On the day mating treatments were initiated, mean $\pm$ SEM female age was 6.7 ± 0.2 and 6.3 ± 0.3 d for blue/green and gray/brown females, respectively. Female age at mating did not differ as a function of abdominal color ($t = 1.01$, $df = 30$, $P = 0.320$).

Over a period of 21 d at 2- to 3-d intervals after initiation of treatments, each mated female was transferred to a new grapefruit seedling. Seedlings were at approximately the same growth stage and had flush growth (immature leaves as described by Hall and Albrigo 2007) available for oviposition. Seedlings at the three-leaf stage were pruned to one leaf ≈ 4 –6 d before use in experiments, which typically yielded

flush at the ideal stage for oviposition. For each female, nine transfers to new seedlings were made, resulting in nine egg "batches" collected. Any females that died before the sixth batch were excluded from analyses. We collected eight replicates of each of the four pairwise combinations of females and males of gray/brown or blue/green abdominal color ($n = 32$ total sample size).

After transferring females to new seedlings, the number of eggs found on each seedling was counted. Every 2–3 d thereafter for 1 wk, any nymphs that hatched were counted and removed. Seedlings with eggs were held in an environmental chamber as described above under Rearing and Housing of Psyllids, but the vial enclosure was removed and a ring of petrolatum was added to the base of the seedling stem to isolate nymphs on the plant. Egg fertility was calculated as the proportion of eggs laid in a given batch that hatched (i.e., the number of nymphs collected divided by the total number of eggs laid).

Body Mass Experiment. To assess whether psyllid body mass (which is related to abdominal color; Wenninger and Hall 2008a) was affected by transfer to fresh plant material, we performed the following experiments. Virgin adults age 5–7 d of either gray/brown or blue/green abdominal color were obtained from the USHRL colony as described above, and the mass of each psyllid was measured to the nearest 0.01 mg. Before handling, psyllids were anesthetized by exposure to carbon dioxide for 8–10 s. Psyllids were then transferred to a new grapefruit seedling and returned to the environmental chamber (as described above). After 5–6 d, the mass of each psyllid was recorded again in the same manner. We collected 15 replicates for each sex and abdominal color combination, except for gray/brown males in which we were only able to obtain nine individuals. Changes in abdominal color from gray/brown to blue/green after transfer to new plants could not be readily assessed because most females took on an orange/yellow color due to the production of ova (E.J.W., unpublished data; Wenninger and Hall 2008a).

Crushed Female Behavioral Assay. The objective of this study was to determine the effect of psyllid abdominal color on male response to a putative female-produced sex attractant pheromone. Behavioral evidence for a sex attractant in *D. citri* has been established (Wenninger et al. 2008). Separate experiments were conducted to evaluate the response of blue/green males to gray/brown or blue/green females ($n = 30$ males tested to each female morphotype) or gray/brown males ($n = 20$) to each type of female. Mated adult psyllids were obtained from the CREC colony described above 5–10 d after adult emergence, because previous experiments showed that mated females are more attractive to males of either mating status compared with virgin females (Wenninger et al. 2008). All experiments were conducted in closed petri dishes (100 by 15 mm, diameter $\times$ height) containing one piece of Whatman no. 1 filter paper (90 mm in diameter) on the lower surface. The assay was initiated by crushing four females of the

same morphotype near the edge of the filter paper with a glass rod. Female psyllids were crushed so as to fit within a premarked 5-mm-diameter circle. Immediately thereafter, one male psyllid was introduced into the petri dish onto a premarked area 70 mm away from where the females were crushed on the opposite edge of the filter paper, and the petri dish was covered with a translucent lid to begin observations. The assays were conducted under 1,000–1,500 lux in a room at 26°C and 60% RH. Each male psyllid within the petri dish was observed under a stereomicroscope for 15 min or until contacting and subsequently leaving the 5-mm-diameter area of crushed females. The data recorded were 1) percentage of males that moved from the point of insertion, 2) the latency time to first movement by those males leaving the insertion point, 3) percentage of males that contacted crushed females, 4) closest distance of approach of those males that did not contact the crushed females, and 5) time spent contacting crushed females for those males making contact. For those males not contacting females, the closest position in relation to the crushed females was marked on the lid of the petri dish with a felt-tipped pen and measured with a ruler at the end of the 15-min observation period.

Data Analysis. To examine the effects of female and male abdominal color on the number of eggs laid per female per day over each of the nine times that eggs were collected, we used repeated measures analysis of variance (ANOVA) with first order autoregressive covariance structure. The model included the interaction between the two main effects as well as between each main effect and the time factor. The number of eggs laid per day was square-root transformed to achieve normality and equal variance. The effects of treatment and time on egg fertility (arcsine transformed) were analyzed similarly with repeated measures ANOVA. Where ANOVA showed significant differences, Tukey's pairwise comparisons tests were conducted to discriminate among means.

For each sex and abdominal color combination in the body mass experiment, we compared the initial and final mass measurements using paired *t*-tests. We also used *t*-tests to compare the gain in mass between psyllids of different abdominal color, separately for males and females.

We used chi-square tests to compare the percentage of males of a given morphotype moving from the insertion point in response to gray/brown versus blue/green females as well as contacting the area of gray/brown versus blue/green crushed females. To compare the latency time to first movement (of those males responding) and the closest distance of approach (of those not making source contact), in response to gray/brown versus blue/green females, means were compared using *t*-tests. The significance level for all analyses was set at $\alpha = 0.05$. Results are presented as mean $\pm$ SEM. All statistical analyses were performed using SAS (SAS Institute 2002).

Table 1. Repeated measures ANOVAs comparing number of eggs that female *D. citri* laid per day (square-root transformed) or egg fertility (arcsine transformed) as a function of female and male abdominal color over nine successive batches of eggs collected

Source of variation	df ^a	F	P
No. of eggs laid per female per day			
Female abdominal color	1, 43.2	1.5	0.231
Male abdominal color	1, 43.2	0.9	0.339
Successive egg batch collected ^b	8, 184	16.3	<0.0001
Female abdominal color × male abdominal color	1, 46	0.07	0.796
Female abdominal color × egg batch	8, 184	4.2	0.0001
Male abdominal color × egg batch	8, 184	3.8	0.0003
Egg fertility			
Female abdominal color	1, 40.7	0.58	0.452
Male abdominal color	1, 40.4	7.1	0.011
Successive egg batch collected ^b	8, 183	11.8	<0.0001
Female abdominal color × male abdominal color	1, 42.6	0.03	0.857
Female abdominal color × egg batch	8, 183	0.44	0.896
Male abdominal color × egg batch	8, 182	1.1	0.371

^a PROC MIXED in SAS may use an approximation of the denominator df, which may result in df given with decimal places.

^b Eggs were collected at nine 2- to 3-d intervals after mating.

Results

Mating Experiment. The number of eggs that females laid per day did not differ as a function of either male or female abdominal color alone nor by the interaction between the main effects; however, fecundity varied significantly as a function of the successive egg batch collected (the time factor in the repeated measures analysis) and the interaction between each main effect and the time factor (Table 1). Specifically, blue/green females laid significantly more eggs per day than did gray/brown females over the first two batches of eggs collected, but thereafter the differences were not significant (Fig. 1). Gray/brown males yielded significantly higher fecundity in their mates in the second batch of eggs collected, whereas blue/green males yielded significantly higher

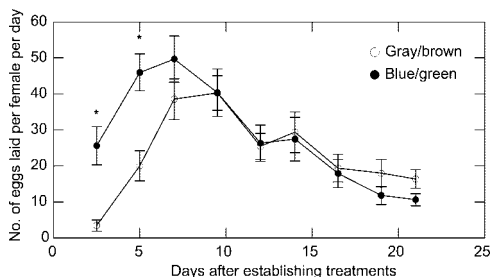


Fig. 1. Mean number of eggs laid per female per day over the nine successive egg batches collected, separated by female abdominal color. Females at 4–8 d of age were paired with a male for 48 h, and eggs were collected at 2- to 3-d intervals after mating. Data were square-root transformed for analysis, but untransformed values are shown here. Error bars represent standard error. For a given batch of eggs collected, an asterisk indicates a significant difference in mean eggs laid per female per day between individuals of gray/brown and blue/green abdominal color. See Table 1 for full analysis.

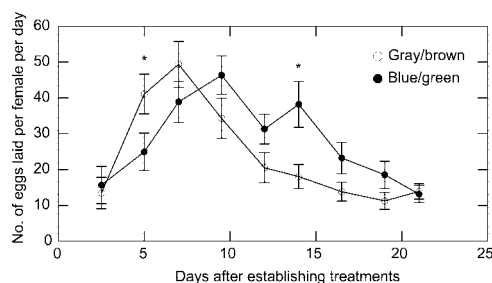


Fig. 2. Mean number of eggs laid per female per day over the nine successive egg batches collected, separated by male abdominal color. Additional notes as in Fig. 1.

fecundity in the sixth batch of eggs collected; the number of eggs that females laid did not differ significantly between females mated to gray/brown versus blue/green males for any of the other batches (Fig. 2). Overall, gray/brown males yielded an earlier, sharp peak in fecundity in their mates with a decline thereafter, whereas females mated to blue/green males showed a more gradual rise and fall in fecundity (Fig. 2).

Egg fertility did not differ according to female abdominal color (Fig. 3), nor were any of the interaction terms significant (Table 1). Instead, egg fertility varied significantly over time and was also significantly affected by male abdominal color (Table 1; Fig. 4). Total egg fertility over the 21-d observation period was significantly higher for females that mated with blue/green males ($65.1 \pm 3.4\%$) relative to females that mated with gray/brown males ($47.8 \pm 3.6\%$; Table 1). Regardless of psyllid abdominal color, egg fertility remained around 80% through the third or fourth batches of eggs collected (≈ 7 – 10 d after establishing treatments) and then declined (Figs. 3–4). However, egg fertility declined more gradually in females mated to blue/green males (Fig. 4).

Body Mass Experiment. Both gray/brown and blue/green females showed a significant increase in body mass after transfer to new seedlings (Table 2); however, the difference between final and initial mass measurements was significantly ($t = 3.32$, $df = 28$, $P = 0.003$) greater for gray/brown females (0.172 ± 0.03 mg; mean 37.2% increase) than for blue/green females

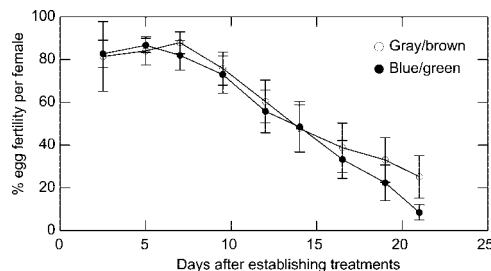


Fig. 3. Mean egg fertility per female over the nine successive egg batches collected, separated by female abdominal color. Data were arcsine transformed for analysis, but untransformed values are shown here. Additional notes as in Fig. 1.

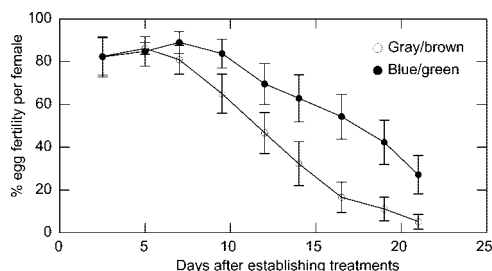


Fig. 4. Mean egg fertility per female over the nine successive egg batches collected, separated by male abdominal color. Additional notes as in Figs. 1 and 3.

(0.057 ± 0.02 mg; mean 11.1% increase). Gray/brown males also showed a significant increase in body mass after transfer to new seedlings, but blue/green males did not (Table 2). For males, the difference between final and initial mass measurements was not significant ($t = 1.71$, $df = 22$, $P = 0.102$) for gray/brown (0.053 ± 0.01 mg; mean 12.4% increase) versus blue/green (0.021 ± 0.01 mg; mean 4.6% increase) individuals.

Crushed Female Behavioral Assay. Overall, responses were similar for gray/brown and blue/green males, with males showing evidence of greater attraction to blue/green females than gray/brown females. Every male that we observed in the blue/green female assays moved from the point of insertion, whereas a smaller percentage of males moved when assayed with gray/brown females; however, the difference was significant only for gray/brown males (Table 3). For both gray/brown and blue/green males, individuals took significantly longer to begin moving (of those that did move during the observation period) when assayed with gray/brown females (Table 3). For both male color categories, a significantly higher percentage of males contacted the crushed females when assayed with blue/green females; none of the males observed made contact with crushed gray/brown females (Table 3). Gray/brown males spent 7.4 ± 2.6 s in contact with crushed blue/green females, and blue/green males spent 8.4 ± 2.1 s in contact with crushed blue/green females. Of the males that did not make contact with crushed females, males moved significantly closer to females when assayed with blue/green females compared with males assayed with gray/brown females (Table 3).

Discussion

Here, we show that individual variation in abdominal color in *D. citri* is related to reproductive output in both males and females as well as to the apparent attractiveness of females to males. For both sexes, gray/brown individuals exhibited lower reproductive output relative to blue/green individuals. However, the specific relationships between abdominal color and reproductive output differed markedly between the sexes.

Gray/brown females laid fewer eggs in the first days after mating, but ≈ 1 wk after being paired with a male, the number of eggs that females laid did not differ between gray/brown and blue/green females. Overall fecundity did not differ according to female abdominal color over the course of the experiment, and all females were laying few eggs per day by the end of our 21-d observation period, suggesting that continued observations would not yield further differences in fecundity. Egg fertility did not differ as a function of female abdominal color, but it did decline over the observation period. The declines in fecundity and egg fertility after a brief pairing with one male are consistent with previous data showing that female *D. citri* require multiple matings to maintain high reproductive output (Wenninger and Hall 2008b). The body mass experiment was conducted to test whether the sharp increase in fecundity of gray/brown females ≈ 1 wk after pairing was related to being transferred to a new plant every 2–3 d, which was necessary given the high number of eggs that most females laid. Both gray/brown and blue/green females showed an increase in body mass, which was almost certainly due at least in part to the production of ova. However, gray/brown females gained more mass than blue/green females, suggesting that they may have been adding body mass from feeding on new plant material and/or were producing more ova than blue/green females—presumably having started with fewer ova.

In the aphid *Sitobion avenae* (F.), which apparently exhibits hereditarily stable, genetically based color variation, developmental and population growth rates varied between different color morphs (Araya et al. 1996). Hougardy and Mills (2008) reported slight differences in reproductive output in different color morphs in the walnut aphid, *Chromaphis juglandicola* (Kaltenbach), but the underlying causes a color variation in this species remain to be investigated. Data on

Table 2. Comparisons of initial and final (after 5–6 d) mass of individual female or male *D. citri* of either gray/brown or blue/green abdominal color when transferred to a new grapefruit seedling after the initial mass measurement

Sex	Abdominal color ^a	n	Mean $\pm$ SEM (mg)		<i>t</i> ^b	P
			Initial mass	Final mass		
Female	Gray/brown	15	0.487 ± 0.02	0.659 ± 0.02	6.69	<0.0001
Female	Blue/green	15	0.550 ± 0.02	0.607 ± 0.03	2.49	0.026
Male	Gray/brown	9	0.437 ± 0.02	0.490 ± 0.02	3.72	0.006
Male	Blue/green	15	0.478 ± 0.01	0.499 ± 0.01	1.72	0.108

^a Represents initial abdominal color, which may have changed before final mass measurement was recorded.

^b Initial and final mass measurements were compared using paired *t*-tests.

Table 3. Comparisons of mean responses in petri dish assays of individual male *D. citri* (separated by abdominal color) to crushed females of either gray/brown or blue/green abdominal color

Variable	Mean $\pm$ SEM		Test statistic	<i>P</i>
	Gray/brown females	Blue/green females		
Gray/brown males				
% of males that moved from insertion point	40.0	100.0	$\chi^2 = 5.1$	0.02
Time (s) to first movement (of males that moved)	625.9 $\pm$ 146.5	321.7 $\pm$ 97.3	$t = 4.0$	0.09
% of males that contacted females	0.0	40.0	$\chi^2 = 9.0$	0.003
Closest distance (mm) of males that did not contact females	47.2 $\pm$ 4.5	15.2 $\pm$ 3.8	$t = 26.4$	0.001
Blue/green males				
% of males that moved from insertion point	67.7	100.0	$\chi^2 = 2.0$	0.157
Time (s) to first movement (of males that moved)	606.1 $\pm$ 71.6	246.3 $\pm$ 51.6	$t = 18.5$	<0.0001
% of males that contacted females	0.0	53.3	$\chi^2 = 16.0$	<0.0001
Closest distance (mm) of males that did not contact females	39.4 $\pm$ 5.0	19.6 $\pm$ 4.5	$t = 8.2$	0.007

$n = 20$ for gray/brown males and $n = 30$ for blue/green males.

individual variation in body color in insects are scarce, but body color in *Aphis gossypii* Glover was shown to be correlated with nitrogen fertilization regimes of host plants (Nevo and Coll 2001). All seedlings used in our experiments were under the same fertilization regime and were matched as far as possible for size, degree of flush, and overall visually observable quality, so—although it is clear that gray/brown females benefited from being transferred to a new plant—it remains uncertain whether variation in plant quality plays a role in influencing abdominal color and body mass in *D. citri*. Complementary experiments in which the mass was recorded in gray/brown females that were maintained on their initial seedling rather than being transferred to new seedlings were unsuccessful because most females died before the second mass measurement could be recorded (E.J.W., unpublished data). These data suggest that psyllids may be negatively affected by continuous feeding on the same seedling, though whether gray/brown abdominal color is the result of continuous feeding on a seedling or gray/brown individuals are more negatively affected by continuous feeding on a seedling remains to be clarified. It is noteworthy that gray/brown adult females generated from our rearing system (described under Rearing and Housing Psyllids) were relatively rare ($\approx 5\%$), but as many as 50% of adults collected from the field or directly from our laboratory colony may be gray/brown (Wenninger and Hall 2008a; E.J.W., unpublished data). We speculate that high densities of immature psyllids on a plant may reduce plant quality—at least locally on a given shoot—resulting in a higher proportion of individuals that exhibit gray/brown abdominal color as adults. Feeding damage caused by immature and adult *D. citri* results in malformation or complete abscission of shoots and leaves (Michaud 2004), presumably related to the injection of salivary toxins, as has been suggested for other hemipteran species (e.g., Backus et al. 2005, Liu et al. 2006, Nissinen et al. 2007). Other factors, including endosymbionts, genetic predisposition, or plant micronutrient levels might contribute as well.

The differences in reproductive output between gray/brown and blue/green males were more complex than for females. Females mated with gray/

brown males showed an earlier, sharper peak in fecundity as well as lower total egg fertility relative to females mated with blue/green males. These patterns are reminiscent of different sperm precedence strategies in which a male (in this case, a gray/brown male) might transfer more ovulation stimulants, oviposition stimulants, and/or other accessory gland products in his ejaculate that enhance his relative fertilization success at the expense of the female's overall fitness (Chapman et al. 1995, Simmons 2001, Gillet 2003). Given that females require multiple matings to achieve high reproductive output (Wenninger and Hall 2008b), it may be advantageous for a male to stimulate a high oviposition rate in his mate before she mates with additional males. How these different proposed reproductive strategies might be related to male abdominal color is unknown. Gray/brown males gained mass after being transferred to a new seedling, suggesting that gray/brown males may have lower nutrient reserves and, therefore, also benefit from feeding on fresh plant material, as is the case with gray/brown females.

Irrespective of male abdominal color, males responded differently to gray/brown versus blue/green females. When assayed with blue/green females, males were generally more likely to move from their point of insertion in the petri dish and showed a shorter latency to first movement. Males also moved closer to females and were more likely to make contact with females when assayed with blue/green females. Wenninger et al. (2008) found behavioral evidence for a female-produced sex attractant, and the data presented here suggest that the putative sex pheromone may be produced in greater quantities by blue/green relative to gray/brown females. Given the lower initial fecundity of gray/brown females observed here, it may not be surprising that males would show greater evidence of attraction to blue/green females. Future work aimed at identifying the putative sex pheromone of *D. citri* should focus on mated females (see Wenninger et al. 2008) and/or females of blue/green abdominal color.

The data presented here show that the color variants observed in *D. citri* by Husain and Nath (1927), Skelley and Hoy (2004), and Wenninger and Hall

(2008a) represent nontrivial morphological variation. Variance in abdominal color is associated with reproductive output in both sexes and with attractiveness of females to males. Husain and Nath (1927) speculated that differences in abdominal color in *D. citri* reflected the contents of the abdomen, and Wenninger and Hall (2008a) further speculated that abdominal color might be associated with the quantity or quality of food ingested. That gray/brown individuals of both sexes gained mass when transferred to new seedlings suggests that psyllids derived some benefit from the transfer. Future work should be aimed at clarifying the underlying mechanisms of the development and maintenance of gray/brown abdominal color, including the possible role of salivary secretions and/or effects of high local intraspecific competition on host plant quality.

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