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Research

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# Density-Dependent Phenotypic Plasticity in the South American Locust, *Schistocerca cancellata* (Orthoptera: Acrididae)

**Martina E. Pocco,[1,2,5] M. Marta Cigliano,[1,2] Bert Foquet,[3] Carlos E. Lange,[1,4] Eliana L. Nieves,[1] and Hojun Song[3]**

[1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CONICET-UNLP, Boulevard 120 s/n entre av. 60 y calle 64, (1900) La Plata, Argentina, [2]División Entomología, Museo de La Plata, Universidad Nacional de La Plata, (1900) La Plata, Argentina, [3]Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475, [4]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Calle 562 e/10 y 11, (1900) La Plata, Argentina, and [5]Corresponding author, e-mail: martinapocco@fcnym.unlp.edu.ar

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## Abstract

Historically, the South American locust, *Schistocerca cancellata* (Serville, 1838), has been considered the most serious agricultural pest in Argentina. An outbreak of a magnitude not recorded since 1954 started in 2015 through 2017 in northern Argentina and neighboring Paraguay and Bolivia. *Schistocerca cancellata* is widely considered as a true locust, with pronounced locust phase polyphenism, although the expression of its phenotypic plasticity has never been quantitatively tested under different density conditions. In this study, we explicitly quantified density-dependent reaction norms in behavior, coloration, and morphology in last instar nymphs of *S. cancellata* under isolated and crowded conditions. We also quantified density-dependent plasticity in adults (size) and in some life history traits. Our results showed that crowded nymphs were significantly more active and more attracted to congeners than isolated nymphs, and developed a much higher percentage of black pattern color. We also found that density had strong effects on body size and there was a sex-dependent pattern in both nymphs and adults, revealing that differences in size between males and females were less pronounced in crowded locusts. We have recorded for the isolated nymphs the presence of about 50% more hairs in the hind femora than in crowded nymphs. Finally, the mean duration of each nymphal instar and adult stage was significantly longer in isolated individuals. We have found strong resemblance with the desert locust, *S. gregaria* (Forskål, 1775) in several traits, and we conclude that *S. cancellata* exhibits an extreme form of density-dependent phenotypic plasticity in behavior, coloration, morphology, and life history traits.

**Key words:** Argentina, locust, South America, phase polyphenism

The South American locust, *Schistocerca cancellata* (Serville, 1838), has been historically considered as the most serious agricultural pest in Argentina (Kohler 1962, Gastón 1969). It is the only swarming locust in southern South America, with its maximum known invasion area covering nearly 4,000,000 km$^2$ affecting central and northern Argentina, Uruguay, southern Brazil, Paraguay, southeast Bolivia, and central and northern Chile. The plagues of *S. cancellata* in Argentina were well-documented from 1870s to 1954, with regular plagues affecting 15 of Argentina's 22 provinces (Hunter and Cosenzo 1990). Similar records were found from neighboring countries, and it is reported that these countries were affected only in years when a plague already existed in Argentina (Hunter and Cosenzo 1990). The last major plague in the 20th century took place between 1943 and 1955 (Gastón 1969, Waloff and Pedgley 1986). From mid last century, there have often been infestations of bands and swarms, especially in the northwestern regions in Argentina (La Rioja, Catamarca, and San Luis provinces) (De Wysiecki and Lange 2005), but extensive campaigns using chemical pesticides to control hopper bands in the agricultural lands have apparently prevented the development of large plagues, so much so that there were no major plagues between 1954 and 2015. However, in late 2015 and January 2016, northern Argentina was massively hit by a plague of *S. cancellata*, which was the worst of its kind in more than half a century for this country. According to Argentina's SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria), more than 100 outbreaks have affected >700,000 ha in 2016 alone.

*Schistocerca cancellata* is one of about 20 species of grasshoppers that are considered true locusts. Locusts are grasshoppers (Orthoptera: Acrididae) that can form dense groups (bands of hoppers and/or swarms of adults which migrate) through an extreme form of density-dependent phenotypic plasticity, in which cryptically colored, shy individuals (solitarious phase) can transform into conspicuously colored, gregarious individuals (gregarious phase) in response to increases in population density (Uvarov 1966, Pener 1983). Locusts also exhibit physiological, morphometric, molecular, and ecological differences depending on local population density (Pener 1991; Pener and Yerushalmi 1998; Simpson et al. 1999, 2002, 2005; Tanaka 2001, 2006; Kang et al. 2004; Hassanali et al. 2005; De Loof et al. 2006; Pener and Simpson 2009; Simpson and Sword 2009; Cullen et al. 2017) and this syndrome of coordinated changes is known as locust phase polyphenism (Uvarov 1966, Pener 1991, Pener and Simpson 2009). The South American locust belongs to the same genus as the infamous desert locust, *S. gregaria* (Forskål, 1775), in North Africa and the Middle East and the Central American locust, *S. piceifrons* (Walker, 1870), in Mexico and Central America. A recent phylogenetic study of the genus *Schistocerca* suggested that *S. gregaria* is the earliest diverging lineage within the genus, implying that the presence of locust phase polyphenism, which is extensively characterized in this species (Pener and Simpson 2009), is an ancestral trait for the genus (Song et al. 2017). The majority of *Schistocerca* species do not swarm and *S. cancellata* and *S. piceifrons* do not form a monophyletic group, which suggests that locust phase polyphenism has been lost and regained at least twice throughout the diversification of *Schistocerca* (Song et al. 2017).

According to the available literature, *S. cancellata* is known to exhibit pronounced locust phase polyphenism, including changes in behavior and coloration in response to crowding (Bruch 1936, Harvey 1981). However, these earlier studies only provided qualitative evidence of phase, and it is still unclear how *S. cancellata* responds to the change in density. Because of the 60-yr absence of major plagues in South America, there is virtually no modern study available that has quantified the effect of rearing density in *S. cancellata*. But now, the availability of a lab colony of *S. cancellata* enabled us to conduct rearing experiments to explicitly quantify the effect of rearing density in many traits that are associated with locust phase polyphenism. Furthermore, the behavioral assay technique originally developed for *S. gregaria* (Roessingh et al. 1993), which has been subsequently used for other locust species (Gray et al. 2009, Cullen et al. 2010) as well as some nonswarming *Schistocerca* species (Sword 2003, Gotham and Song 2013), has never been applied to *S. cancellata*. This behavioral assay allows quantifying the behavioral parameters that individual locusts display in response to a density stimulus, thereby providing a more in-depth look into density-dependent behavioral plasticity (Cullen et al. 2012). Because *S. gregaria* behavior has been extensively quantified using this method (Simpson et al. 1999, Cullen et al. 2012), its application to *S. cancellata* will provide an exciting comparative framework to test how similar or different behavioral plasticity is between the two divergent locust species in the same genus (Song 2011). Although documented information on the biology and formation of swarms of *S. cancellata* exists (Gastón 1969, Kohler 1979, Waloff and Pedgley 1986, Hunter and Cosenzo 1990, Sanchez et al. 1997), quantitative studies on its life history parameters are scarce (Barrera and Turk 1983, Sanchez et al. 1997) and none of these studies included rearing locusts at different density conditions. Therefore, studies of the biology of *S. cancellata* including explicit experiments, reared in both isolated and crowded conditions in a controlled manner, are critically needed to fill in the gap in the knowledge of the South American locust.

In this study, we quantify density-dependent phenotypic plasticity in behavior, coloration, and morphology in *S. cancellata* using a laboratory colony established from the individuals collected during the 2016 plague from northern Argentina. We also describe the differences in life history parameters between the locusts reared in isolated and crowded conditions. Because *S. cancellata* is a true locust, we hypothesize that it will show significant differences in all parameters measured when reared in isolated and crowded conditions. We use what is known about locust phase polyphenism in *S. gregaria* as baseline information to compare and contrast how similar or different the two species are.

## Materials and Methods

### Insects and Rearing Conditions

During an outbreak period in March 2016, we visited Catamarca province (Fray Mamerto Esquiú and Capayán Departments), Argentina and collected gregarious nymphs and adults of *S. cancellata* (Fig. 1A–F). In order to quantify density-dependent phenotypic plasticity, we reared the locusts under two density conditions (isolation and crowding) for at least two generations before this study in the facilities of CEPAVE institute. For the isolated treatment, individuals were reared singly in individual cages (for nymphs: 12.5 cm height × 10.5 cm width × 10.5 cm depth; for adults: 25.5 cm height × 10.5 cm width × 10.5 cm depth), designed to prevent visual, tactile, and chemical stimuli from others. For the crowded treatment, about 100 nymphs were reared in each of several wire-screened, aluminum cages (for nymphs: 30 cm height × 22 cm width × 22 cm depth; for adults: 40 cm height × 30 cm width × 30 cm depth). The isolated and the crowded treatments were placed in two separate rooms at 14 h of light and 10 h of darkness, 30°C, and 40% RH. Locusts were fed daily fresh lettuce, wheat bran, and cabbage.

### Quantification of Phenotypic Plasticity

The effect of rearing density in terms of behavior, color, and morphology was quantified in last instar nymphs (3 d after molting) of *S. cancellata* reared in two density treatments. The methodology for the quantification of behavior follows Roessingh et al. (1993) and Gotham and Song (2013), and for the quantification of color and morphology is based on Gotham and Song (2013), who studied the expression of density-dependent phenotypic plasticity in nonswarming *Schistocerca* species.

### Quantification of Nymphal Behavior

The behavioral assay arena designed by Roessingh et al. (1993) and modified by Gotham and Song (2013) was used in this study to quantify behavioral plasticity. The arena was placed in a room at 30°C and 40% RH. The arena (57cm × 31 cm × 11 cm) had a stimulus chamber at each end, one simulating a low-density condition (no locusts) and the other simulating a high-density condition (50 last instar nymphs reared in crowded conditions). A test subject (a locust reared in either density condition) was placed into a blackened 50 ml Falcon tube for 2 min to recover from handling, and then introduced through a small hole in the center of the arena floor. Its behavior in response to the stimulus chambers was recorded during 12 min using a video camera Panasonic HC-V380 positioned directly above the arena. A total of 52 isolated and 56 crowded nymphs were assayed.

The software EthoVision XT (Noldus) was used to analyze the recorded videos of the test locusts. Only the first 10 min of each video were considered for the analysis. In order to measure the

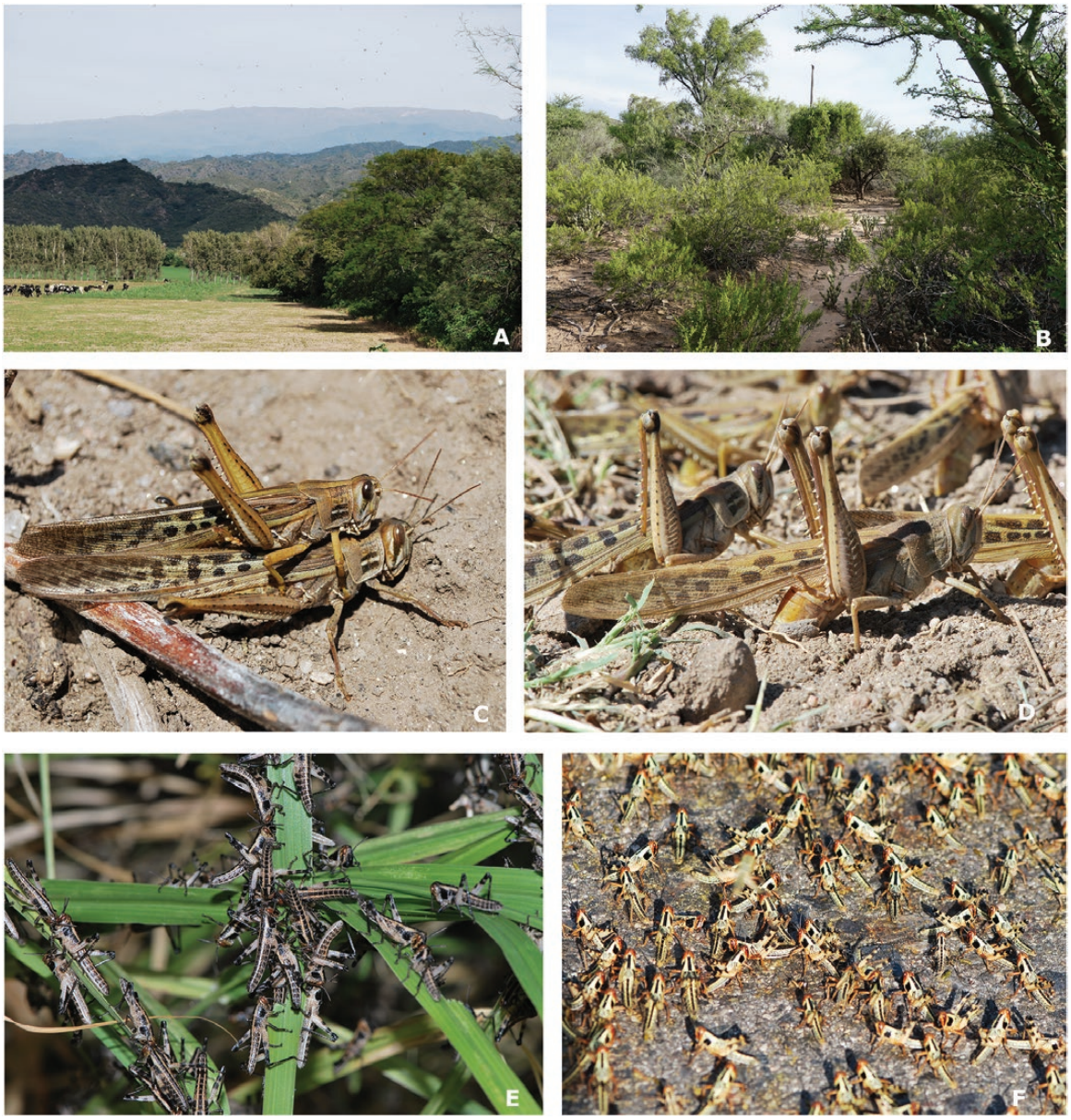


**Fig. 1.** (A and B) Typical locust habitats Catamarca province, during an outbreak of *Schistocerca cancellata*, March 2016. (C–F) Gregarious phase of *S. cancellata*. (C) Male and female mating. (D) Group of females, oviposition. (E) Nymphs at second instar. (F) Nymphs at penultimate instar.

locust's behavior in response to the stimuli, we divided the arena into three zones using the EthoVision software: a stimulus zone (adjacent to the stimulus chamber), a nonstimulus zone (adjacent to the nonstimulus chamber), and a neutral zone (between the two stimulus chambers). To detect the locust in EthoVision, the subject was set as darker than the background, with the sensitivity value set at 35 and the background change was set at medium. Subject contours were first dilated by four pixels and then eroded by two pixels. Minimum and maximum subject sizes were set using default parameters. Raw position data were acquired and all tracks were manually inspected and corrected where necessary and the 'Minimum Distance Moved' (MDM) filter, which removes inherent noises from the tracks, was used to smooth the tracks before exporting the data, following the procedure described in Cullen et al. (2012). The optimum MDM filter chosen for *S. cancellata* was 0.1 cm. For our study, we used a total of eight behavioral parameters, four of which were related to the activity of the locust (Distance moved; Movement; Velocity [calculated as distance moved/movement]; and Total time spent climbing walls), and the remaining four were related to the attraction of the locust to the stimuli (Time spent in stimulus zone; Time spent in nonstimulus zone; Time spent in neutral zone; and Time spent on stimulus wall).

## Quantification of Nymphal Color and Size

After the behavioral assay was completed, the test individuals were placed in Falcon tubes and frozen in a −30°C freezer to preserve coloration. In order to quantify density-dependent color plasticity, individuals removed from the freezer were thawed and a high-resolution digital image of each specimen was captured using a Canon EOS Rebel digital camera. Images of a dorsal view of head, a dorsal view of pronotum, a lateral view of thorax (including pronotum and wing pad), and a lateral view of hind femur were taken. Two attributes of color were measured from the captured images: background color

and black patterns. The background color refers to the baseline coloration of the cuticles in different body parts, and the black patterns refer to distinct black patches that develop in response to crowding.

In order to measure background color from the captured images, a square area (1 mm x 1 mm) on the fastigium of head and another square area on the disk of pronotum (dorsal surface) that had uniform color were defined (Fig. 2B and C). These squares were cropped and saved as individual image files, and then the RGB component values of these squares were measured in ImageJ64 (Rasband 1997–2012). We calculated median RGB values for the crowded and isolated treatments for each structure. Then, we converted the median RGB values to CIELAB values using the online converter at https://www.nixsensor.com/free-colorconverter/. Using the converted CIELAB values, we calculated Delta-E value (CIE 2000 formula) between the isolated and crowded conditions for each structure using the online calculator at http://www.brucelindbloom.com/index.html?ColorDifferenceCalc.html. Delta-E (ΔE) is a numeric value that represents the 'distance' between two colors perceived by human eyes (Brainard 2003). A Delta-E value <1 means imperceptible difference; 1–2, difference through close observation; 2–10, perceptible difference at a glance; 11–49, colors are more similar than opposite; and 100, colors are exact opposite. Although Delta-E is not a statistical comparison of colors, it is the most widely used method for comparing colors.

To measure the amount of black patterns we analyzed the lateral view of pronotum, the lateral view of the wing pad, and the lateral view of the hind femur. We used the polygon tool in ImageJ64 (Rasband 1997–2012) to crop the outlined structures of interest, and saved as image files (Fig. 2A). We measured the total area of each structure, and then we changed the color threshold of each image to red, and we adjusted the brightness in order to select the background coloration. Then we calculated the proportion of black pattern of each structure by subtracting the area with the background coloration from the total area, then dividing the area with black patterns by the total area of the structure.

The high-resolution digital images were also used to quantify morphology. A ruler tool in ImageJ64 (Rasband 1997–2012) was used to measure the linear length of the pronotum and the length of the hind femur. For each structure, we made three measurements and then we calculated an average value, which were used for subsequent statistical analyses. In addition, the number of mechanosensory hairs located on the outer surface of the hind femur which have been implicated in the detection of stimuli associated with crowding (Simpson et al. 2001, Rogers et al. 2003) was counted under a stereomicroscope for each individual, and we calculated the ratio between the number of hairs and the hind femur length.

## Quantification of Adult Morphometrics

Adult individuals from the isolated and crowded treatments were measured using a digital caliper. Five linear measurements were considered (in mm): Body length (BL); Pronotum length (PL); Tegmen length (E); Hind femur length (F); and Head width (C). We calculated the ratios most widely used in phase-related morphometry of locusts: F/C (length of the hind femur/maximum width of the head) and E/F (length of the tegmen/length of the hind femur) (Dirsh 1951, 1953; Uvarov 1966).

## Description and Mean Duration of Nymphal Instars and Adult Stage

Mean duration of stages (life span) was calculated after the second generation of the rearing individuals. The mean duration of each nymphal instar and adult stage (period from the last nymphal molt to death) was calculated for the isolated condition (Instar I: $N = 139$, II: $N = 127$, III: $N = 121$, IV: $N = 67$, V: $N = 67$, VI: $N = 38$, Adults: $N = 61$) and for the crowded condition (Instar I: $N = 200$, Instar II:

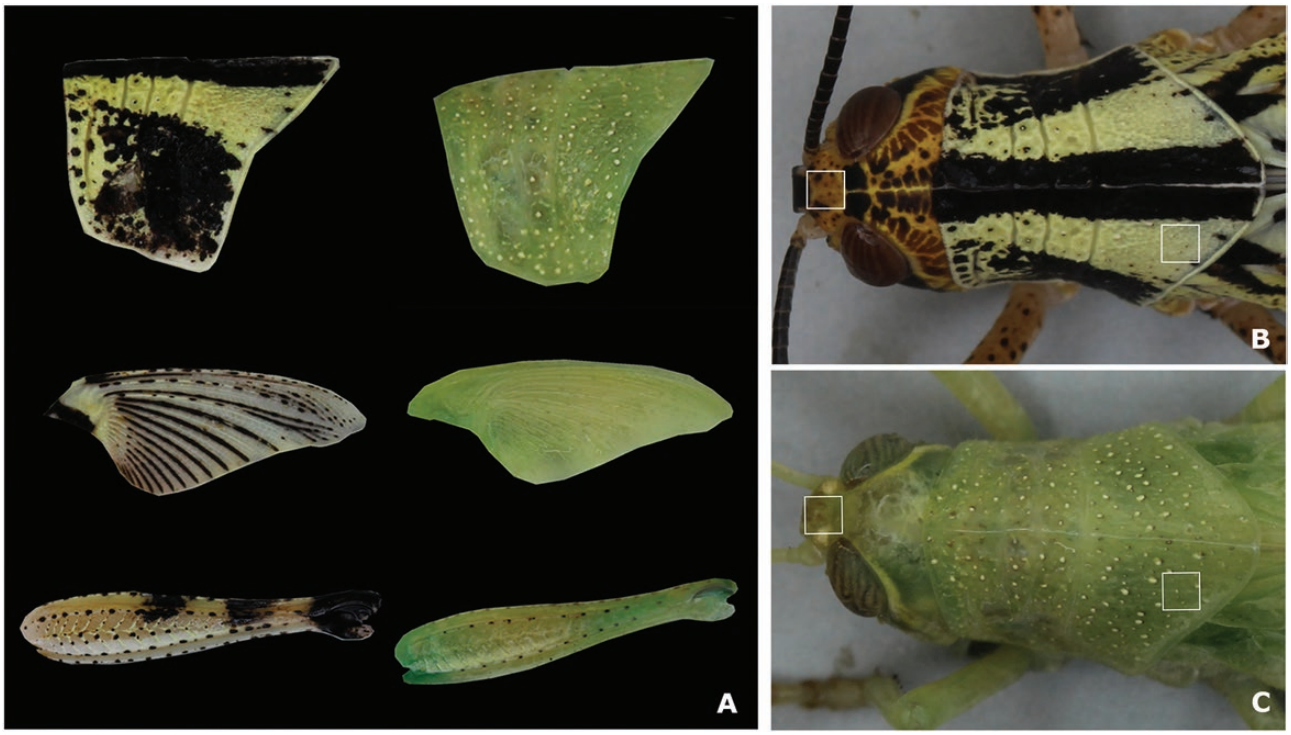


**Fig. 2.** *Schistocerca cancellata.* Structures of the body measured for quantification of color: (A) black pattern in pronotum, wing pad, and hind femur in crowded (left) and isolated nymphs (right). (B and C) Background color in head and pronotum in crowded (B) and isolated nymphs (C).

$N$ = 200, Instar III: $N$ = 190, Instar IV: $N$ = 200, Instar V: $N$ = 180, Instar VI: $N$ = 170, Adults: $N$ = 191). We also photographed the representative specimens of each nymphal instar reared under isolated and crowded conditions using a Nikon D60 digital camera.

### Statistical Analyses

To study the effect of density and sex on the overall behavior, color, and morphology of the last instar nymphs and on the size of adults we used MANOVA. Dependent variables highly correlated (>0.75 or <–0.75) were identified and removed for analysis; also Mahalanobis distance was used to remove multivariate outliers. The behavioral variable Velocity was also removed for MANOVA due to the nonlinear relationship with the remaining variables.

Mean differences between the two rearing density conditions on the behavior and color variables of the last instar nymphs, and on the duration of each nymphal instar and adult stage were tested using $Z$-test; for behavior and color, $P$-values were adjusted per family of tests (Benjamini and Hochberg 1995). The effect of rearing density and sex on the morphology (size and number of femur hairs) of nymphs and size of adults was tested using two-way ANOVA. Tukey's HSD test was used when the effect was significant ($\alpha < 0.05$). Statistical analyses were conducted in R (3.5.1.) (R Core Team 2018). In addition, data sets were analyzed with principal component analysis (PCA), using the Pearson correlation coefficient, in PAST v.3.22 (Hammer et al. 2001).

## Results

### Quantification of Density-Dependent Plasticity in Nymphs

We found that the nymphs reared in crowded conditions were more active and more attracted to the conspecifics than those reared in isolation. When the behavioral variables were analyzed simultaneously, there was a significant effect of rearing density regardless of sex (Table 1). There were statistically significant differences between isolated and crowded treatments in terms of distance moved, movement, and time spent on wall climbing (Table 2; Fig. 3A), in which the crowded nymphs moved more distance, moved more, and climbed significantly more over walls than isolated ones. In terms of velocity, however, there were no significant differences between the two conditions. There were also significant differences between isolated and crowded nymphs in terms of time spent on the stimulus wall, time spent in nonstimulus zone as well as in neutral zone (Table 2; Fig. 3A). Crowded nymphs spent more time on the stimulus wall and in the nonstimulus zone, while isolated nymphs spent more time in neutral zone. There were no significant differences in terms of time spent in the stimulus zone between isolated and crowded nymphs, although crowded nymphs tended to spend more time.

Results of PCA performed on the eight behavioral parameters (activity- and attraction-related variables) show that specimens, independently of sex, were distributed in a same cloud, although the crowded specimens were mostly plotted on the right within the multivariate space, while most of the isolated specimens were represented on the left within the cloud (Fig. 3B). The first two components accounted for 72.24% of the total variation (48.78 and 23.46%, respectively) (Supp Table 1 [online only]). In PCI, three activity-related variables (distance moved, movement, and time spent climbing walls) and one attraction-related variable (time spent in neutral zone) were the ones that most contributed to the variation, nearly in equal representation. In PCII, the time spent in nonstimulus zone and in stimulus zone, both attraction-related variables, had the major contribution to the variation of this component (Supp Table 1 [online only]). The results show a clear effect of density in the activity and also in terms of attraction.

The rearing density had a major effect in terms of coloration (Table 2; Fig. 4A). Crowded nymphs had a much higher percentage of black patterns in pronotum, wing pads, and hind femora (Fig. 2A), while isolated nymphs had a low amount of black pattern, restricted to small black dots distributed in variable degree in certain structures (legs, abdomen, wing pads, pronotum) or distributed all over the body. Results of PCA conducted on the black pattern variables show that the three variables (black pattern of pronotum, wing pads, and hind femur) equally contributed to PCI, which accounted for 97.43% of the total variation (Supp Table 1 [online only]). The PCII accounted for 2.00% of the total variation, and in this case the black pattern of wing pads was the variable that most contributed. These results also show a clear effect of density in the black pattern

**Table 1.** MANOVA table showing the effects of rearing density and sex on the overall behavior and morphology of last instar nymphs and size of adults of *Schistocerca cancellata*

| Variable | Source | df | Pillai | Approx. $F$ | Num df | Den df | Pr (>$F$) |
|---|---|---|---|---|---|---|---|
| Behavior[a] | Density | 1 | 0.48748 | 14.7424 | 6 | 93 | <0.001* |
| | Sex | 1 | 0.09547 | 1.636 | 6 | 93 | 0.1459 |
| | Density:sex | 1 | 0.03546 | 0.5699 | 6 | 93 | 0.7533 |
| | Residuals | 98 | | | | | |
| Morphology[b] | Density | 1 | 0.77203 | 172.717 | 2 | 102 | <0.001* |
| | Sex | 1 | 0.77217 | 172.846 | 2 | 102 | <0.001* |
| | Density:sex | 1 | 0.2121 | 13.729 | 2 | 102 | <0.001* |
| | Residuals | 103 | | | | | |
| Size (adults)[c] | Density | 1 | 0.64188 | 141.598 | 3 | 237 | <0.001* |
| | Sex | 1 | 0.74576 | 231.728 | 3 | 237 | <0.001* |
| | Density:sex | 1 | 0.07803 | 6.687 | 3 | 237 | <0.001* |
| | Residuals | 239 | | | | | |

Color was not analyzed due to the high correlation among variables.

[a]Distance moved, time spent on wall climbing, time spent on stimulus wall, time spent in stimulus zone, time spent in nonstimulus zone, time spent in neutral zone.

[b]Hind femur length and number of hairs.

[c]Body length, E/F, and F/C.

*Significant differences.

**Table 2.** Values from *Z*-test (*Z*) showing the differences in behavior and color between isolated and crowded treatments for *Schistocerca cancellata* last instar nymphs

| Behavior | | |
|---|---|---|
| | *Z* | p BH |
| Activity-related parameters | | |
| Distance moved | 9.6090 | <0.001* |
| Movement | 11.1683 | <0.001* |
| Velocity | 1.3515 | 0.2017 |
| Time spent on wall climbing | 3.9143 | <0.001* |
| Attraction-related parameters | | |
| Time spent on stimulus wall | 2.676 | 0.0102* |
| Time spent in stimulus zone | 1.1865 | 0.2354 |
| Time spent in nonstimulus zone | 2.6675 | 0.0102* |
| Time spent in neutral zone | –3.5615 | <0.001* |
| Color | | |
| Black Pattern | *Z* | p BH |
| Pronotum | 90.7550 | <0.001* |
| Wing pad | 30.8240 | <0.001* |
| Hind femur | 56.0936 | <0.001* |

Benjamini–Hochberg adjusted *P*-values (p BH) are indicated.
*Significant differences between the two density conditions.

regardless of sex, evidenced by the two separate clouds corresponding to crowded specimens distributed on the right of the multivariate space, and isolated specimens grouped in close proximity in a cloud on the left (Fig. 4B). In terms of background color, crowded nymphs developed pale orange to orange-red color in the head and cream to yellow in the pronotum, while isolated nymphs had green or light brown color in the head and pronotum. The Delta-E value was 6.138605 for the head and 17.793116 for the pronotum, which indicated that the background coloration of head and pronotum dramatically differed between the two density conditions (Fig. 2B and C). A comparative illustration of the six nymphal instars under isolated and crowded conditions in the laboratory is presented in Fig. 5. In crowded nymphs, the black pattern was evident after a few hours of hatching. The coloration during the first two instars was in general pale; the orange-red color of the head and the bright black and yellow turned more evident after the third instar. For isolated nymphs, most of the individuals displayed a green or light green coloration, with different amounts of black dots. However, a very pale green or light brown coloration was commonly observed. In some occasions, the body presented darker areas in the same areas of black pattern of crowded nymphs (Fig. 5).

The body size was also affected by the rearing density and there were also sex-specific differences (Fig. 6A). We found a significant effect of density, sex, and the interaction between density and sex on the size (Table 3). We found that crowded female nymphs were significantly smaller than isolated ones in terms of pronotum and femur lengths, while crowded male nymphs were significantly larger than isolated ones (Supp Fig. 1, Supp Table 2 [online only]).

Finally, the density had a significant effect in the number of hairs on the outer face of hind femora (Fig. 6A; Table 3). Isolated nymphs had significantly more hairs than crowded nymphs in both sexes (Supp Fig. 1, Supp Table 2 [online only]). We found a significant effect of density, sex, and the interaction between density and sex in the ratio hairs/femur length (Table 3; Supp Fig. 1 [online only]), as well as when variables were analyzed simultaneously (Table 1). Isolated females had 50.3 % more hairs than crowded females, and isolated males had 45.4% more hairs than crowded males. In general, the hairs on the outer face of hind femora in isolated nymphs were lightly colored and longer than those in crowded nymphs.

When the pronotum and the hind femur lengths, the number of hairs, and the ratio hairs/FL were simultaneously analyzed to visualize the effect of density and sex in the groupings of individuals, a clear pattern emerged in the multivariate space (Fig. 6B). A cloud comprising isolated males on the right of the space and a separate cloud comprising isolated females on the upper quadrants were depicted, while crowded specimens were plotted separately onto the left, with males and females being slightly separated. The PCI and PCII accounted for 97.83% of the total variation, both with similar percentage of variance (49.73 and 48.10%, respectively). The ratio hairs/femur length and the number of hairs were the variables that contributed the most to the variation of PCI, while in PCII the pronotum and the hind femur length had the most representation (Supp Table 1 [online only]). These results indicate a clear effect of density and sex in the number of hairs and a sex difference in size more pronounced between isolated specimens than between crowded specimens.

## Quantification of Density-Dependent Plasticity in Adults

The morphometric ratios E/F and F/C and the mean linear measurements obtained for isolated and crowded adults are shown in Table 4. MANOVA analysis conducted on the size variables found a significant effect of density, sex, and the interaction between density and sex on the adult size when analyzed simultaneously (Table 1). The ratio E/F did not differ between the two density conditions, while for the F/C ratio there was a significant effect of density (Table 5). The F/C ratio was significantly higher in isolated adults regardless of sex (Supp Fig. 1, Supp Table 3 [online only]). We found a significant effect of density, sex, and the interaction between density and sex on the adult size (Table 5). Crowded and isolated females had similar body length, tegmen length, and femur length; while the head was shown to be significantly wider and the pronotum slightly larger, but also significantly, in crowded females (Supp Fig. 1, Supp Table 3 [online only]). The differences in size were more conspicuous in the males, resulting in crowded males being significantly larger in body, tegmen, hind femur, and pronotum and with a wider head than isolated males (Supp Fig. 1, Supp Table 3 [online only]). A similar pattern was observed in the results of the PCA performed on the body size. PCI and PCII accounted for a 79.87% of the total variation (59.75 and 20.12%, respectively) (Supp Table 1 [online only]). In PCI, the five linear measurements contributed almost equally to this component, although the body length had a higher representation. On the other hand, in PCII the E/F and F/C ratios were the variables with major contribution. There was a strong effect of sex on the adult size, with the females distributed on the right of the multivariate space and the males on the left. As found for the nymphs, the difference in size between males and females of the crowded condition was less pronounced than the difference between isolated males and females. Regarding the effect of density, a different pattern was found for females and for males. Females were plotted in a same cloud regardless of density, indicating slight differences in size. However, a clear effect of density on the size was found for the males, where isolated specimens were mostly plotted at the left of the diagram.

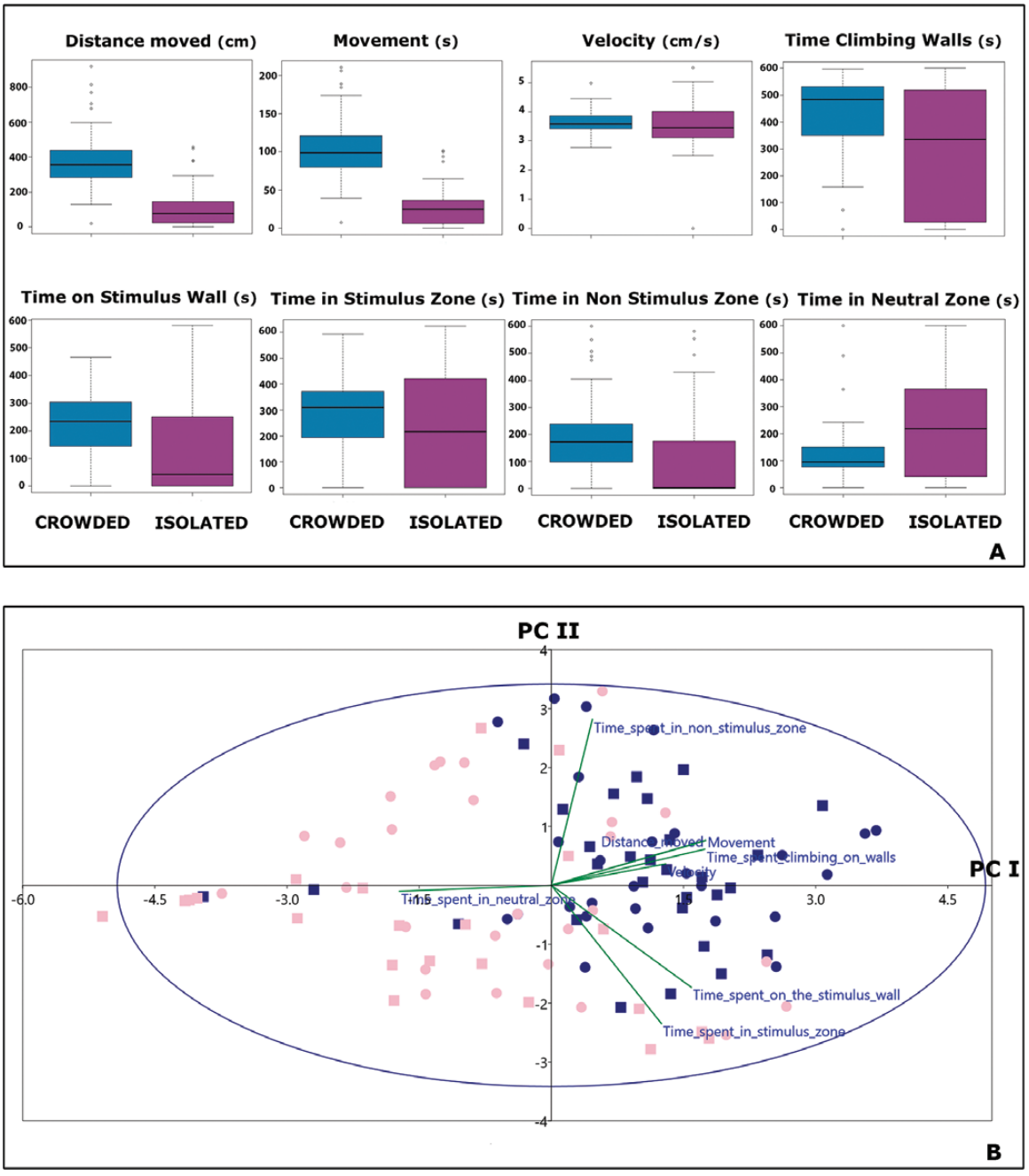


**Fig. 3.** (A) Density-dependent reaction norms in behavior (activity- and attraction-related variables) for last instar nymphs of *Schistocerca cancellata*. Bars as indicated. (B) Biplot showing the results of PCA on the overall behavior (eight behavioral parameters) for *S. cancellata* male (circles) and female (squares) last instar nymphs, in isolated (light color symbols) and crowded (dark color symbols) conditions.

### Quantification of Density-Dependent Plasticity in Life History Traits

In the laboratory, for both density conditions, six nymphal instars were recorded (Table 6). In a few (nine) isolated nymphs, an extra molting (seven instars) occurred. The duration of total nymphal development was 47.92 d for isolated nymphs, and 35.62 d for crowded nymphs. The mean duration of each nymphal instar was significantly longer in isolated locusts than in crowded ones (Table 6). Also, the mean duration of adult stage significantly differed between the isolated treatment (86.6 ± 55.7 d) and the crowded treatment (57.95 ± 17.8 d). For both conditions, the number of eye stripes corresponded with the number of instars (e.g., last instar nymphs have six eye stripes; adults have seven stripes).

## Discussion

Despite the fact that *S. cancellata* is the most devastating locust species in South America (Kohler 1962, Gastón 1969), surprisingly little is known about its expression of locust phase polyphenism. It has certainly been known for a long time that this species can exist in the field either as solitarious or gregarious locusts and that it has classic phase-related characteristics, such as color and morphometric ratios

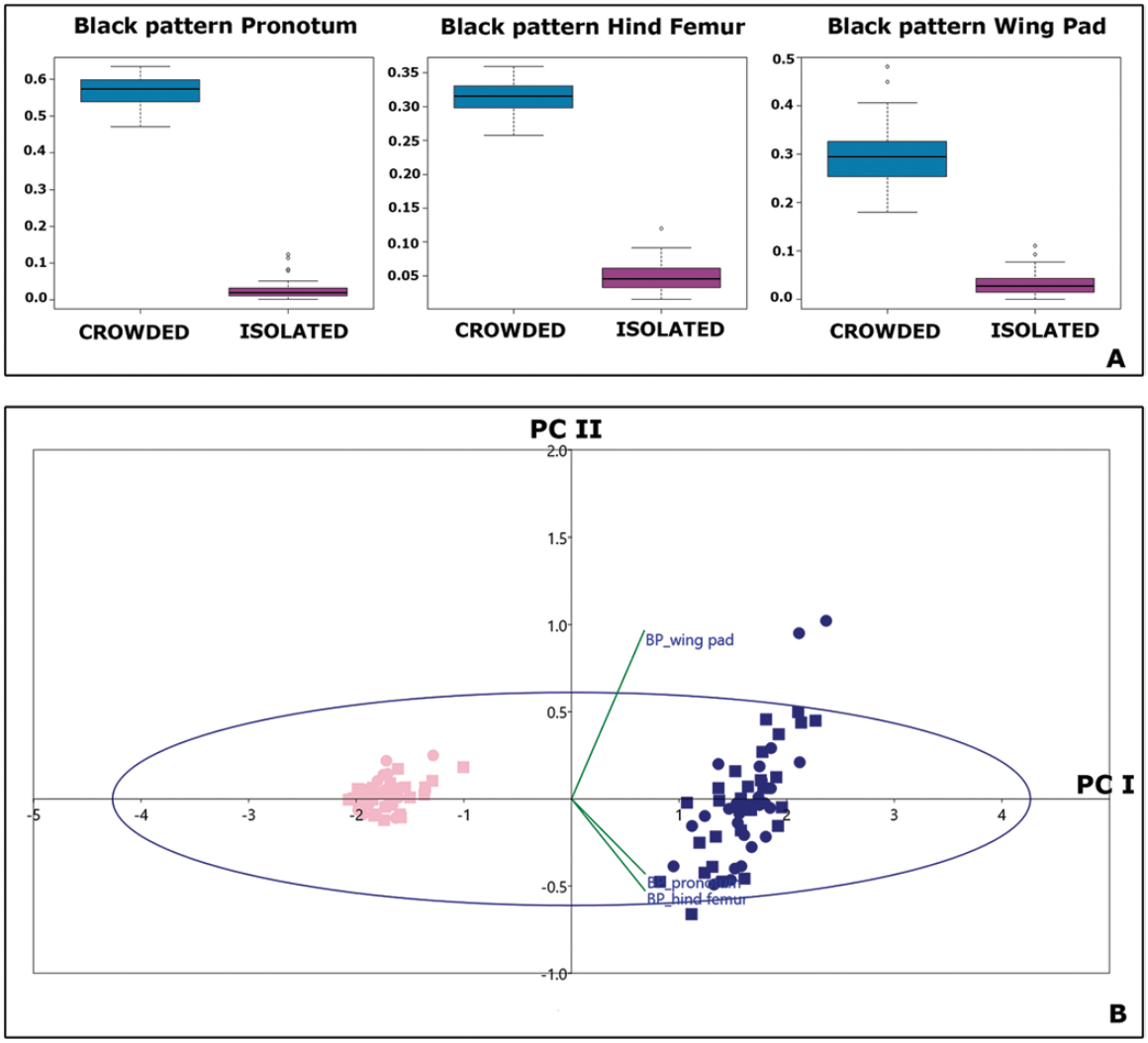


**Fig. 4.** (A) Density-dependent reaction norms in coloration (black pattern) for last instar nymphs of *Schistocerca cancellata*. Bars as indicated. (B) Biplot showing the results of PCA on the coloration (black pattern) for *S. cancellata* male (circles) and female (squares) last instar nymphs, in isolated (light color symbols) and crowded (dark color symbols) conditions.

(Bruch 1936; Bruzzone 1953; Daguerre 1953; Kohler 1962, 1979; Gastón 1969; Harvey 1981; Barrera and Turk 1983). However, such information was often based on field-collected specimens of either extreme phase (Barrera and Turk 1983, Sanchez et al. 1997), rather than based on specific rearing experiments, although there was a study done in the early 20th century, which was mostly qualitative (Bruch 1939). Of course, the 60-yr absence of major plagues in Argentina must have contributed to the decline of studies on *S. cancellata*, but there is a more important reason why this species is understudied. From the early 1990s, the field of locust research went through a period of rejuvenation as scientists started focusing on unraveling the proximate mechanism of phase change using the desert locust (*S. gregaria*) as a model system (Roessingh et al. 1993; Roessingh and Simpson 1994; Roessingh et al. 1998; Simpson et al. 1999, 2001). One particular area of research that received much attention was regarding the mechanisms and the factors contributing to behavioral gregarization and solitarization (Roessingh et al. 1993; Simpson et al. 1999, 2001; Anstey et al. 2009; Rogers et al. 2003). While much of what we know about the behavioral plasticity of locusts comes from the extensive study of *S. gregaria*, as well as the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Oedipodinae), and to a lesser extent, the Australian plague locust, *Chortoicetes terminifera* (Walker, 1870) (Oedipodinae), the fact is that we do not have comparable data from other locust species (Song 2011) and it is not clear that the findings based on *S. gregaria* can be directly applicable to other locust species. Therefore, our study represents the first explicit quantification of density-dependent phenotypic plasticity for another locust species in the genus *Schistocerca* other than *S. gregaria*, and we clearly demonstrate that *S. cancellata* exhibits an extreme form of density-dependent phenotypic plasticity in terms of nymphal behavior, color, size, life history traits, as well as adult morphometric ratios, as expected.

In terms of behavior, our data demonstrate that the behavioral plasticity of *S. cancellata* is quite reminiscent of that of *S. gregaria*. One of the defining characteristics of locust phase polyphenism is the ability to change behavior in response to changes in population density (Pener and Simpson 2009). In *S. gregaria*, experimental studies have shown that the nymphs reared in high density exhibit higher activity- and attraction-related behaviors than those reared in isolation (Roessingh et al. 1993, Anstey et al. 2009, Ott et al. 2012, Rogers et al. 2014). We find that the crowded *S. cancellata* indeed show higher activities and more attraction to conspecifics

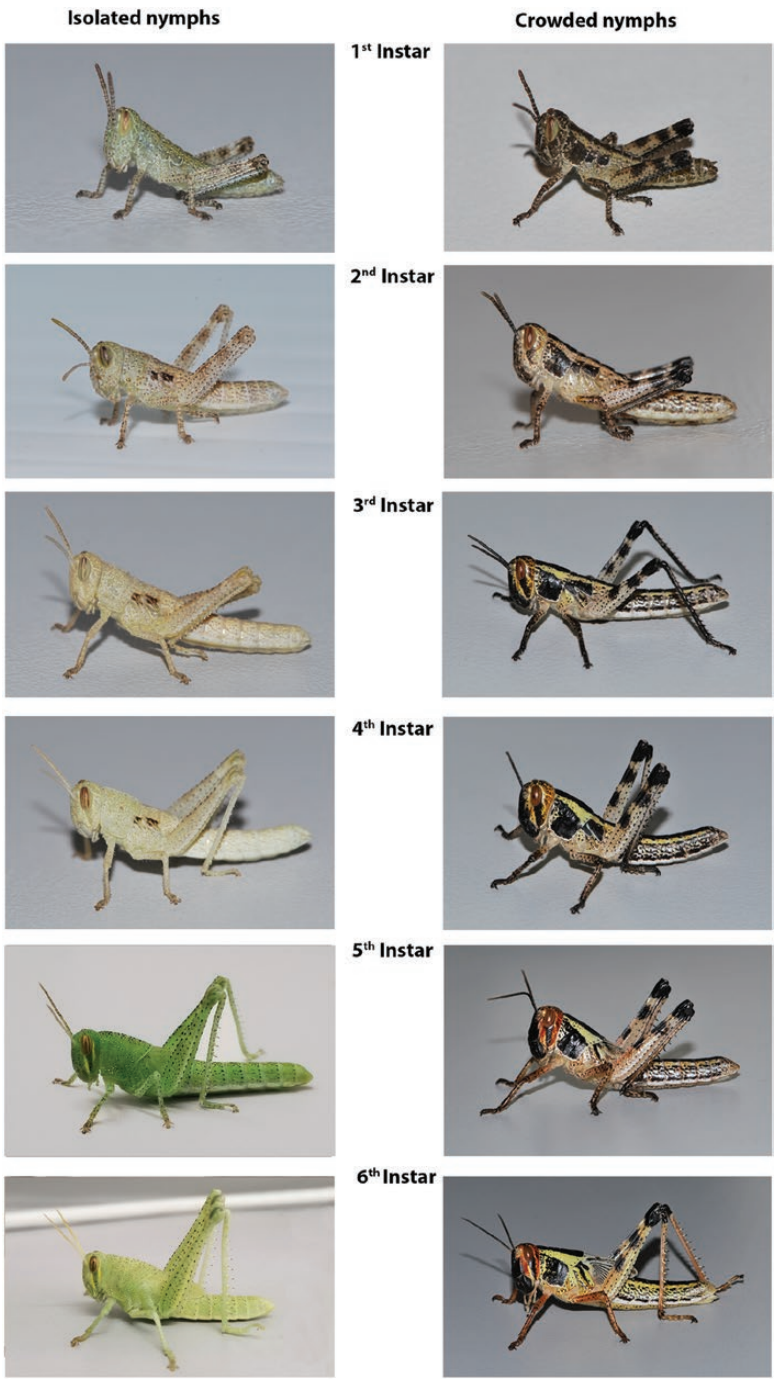


**Fig. 5.** Comparative illustration of the six nymphal instars of *Schistocerca cancellata* under isolated and crowded conditions.

than the isolated individuals, just like *S. gregaria*, and this laboratory finding corresponds well with the observations made in the field. In its natural habitat, solitarious adults of *S. cancellata* behave like other grasshoppers in grasslands. In general, individuals are found dispersed from each other at very low density, usually hidden in the dry vegetation/shrubs, and when disturbed they displace with very fast movements, flying very fast, usually toward higher levels of the vegetation (shrubs/trees) (Pocco, personal observations). Egg pods are laid distant from each other, and after hatching, the resulting nymphs disperse in the vegetation and develop in isolation; they do not move much, feed, and hide in the plants protected by their cryptic coloration (Kohler 1979). When density is high, gregarious nymphs move more and display 'hectic' behavior, with higher individual irritability and more ingestion of food (Kohler 1979). The new adults lay eggs in aggregate groups, on sandy soils (Pocco and Cigliano, personal observations) (Fig. 1D), resulting in multiple egg pods, and after hatching, the nymphs form dense groups in constant movement that eventually constitute bands of marching nymphs (Pocco and Cigliano, personal observations). What the present study demonstrates is that the environmental stimulus inducing plastic responses in behavior in *S. cancellata* is clearly local population density. In *S. gregaria*, the behavioral gregarization occurs rapidly within a few hours of forced crowding or after receiving the appropriate stimuli, but behavioral

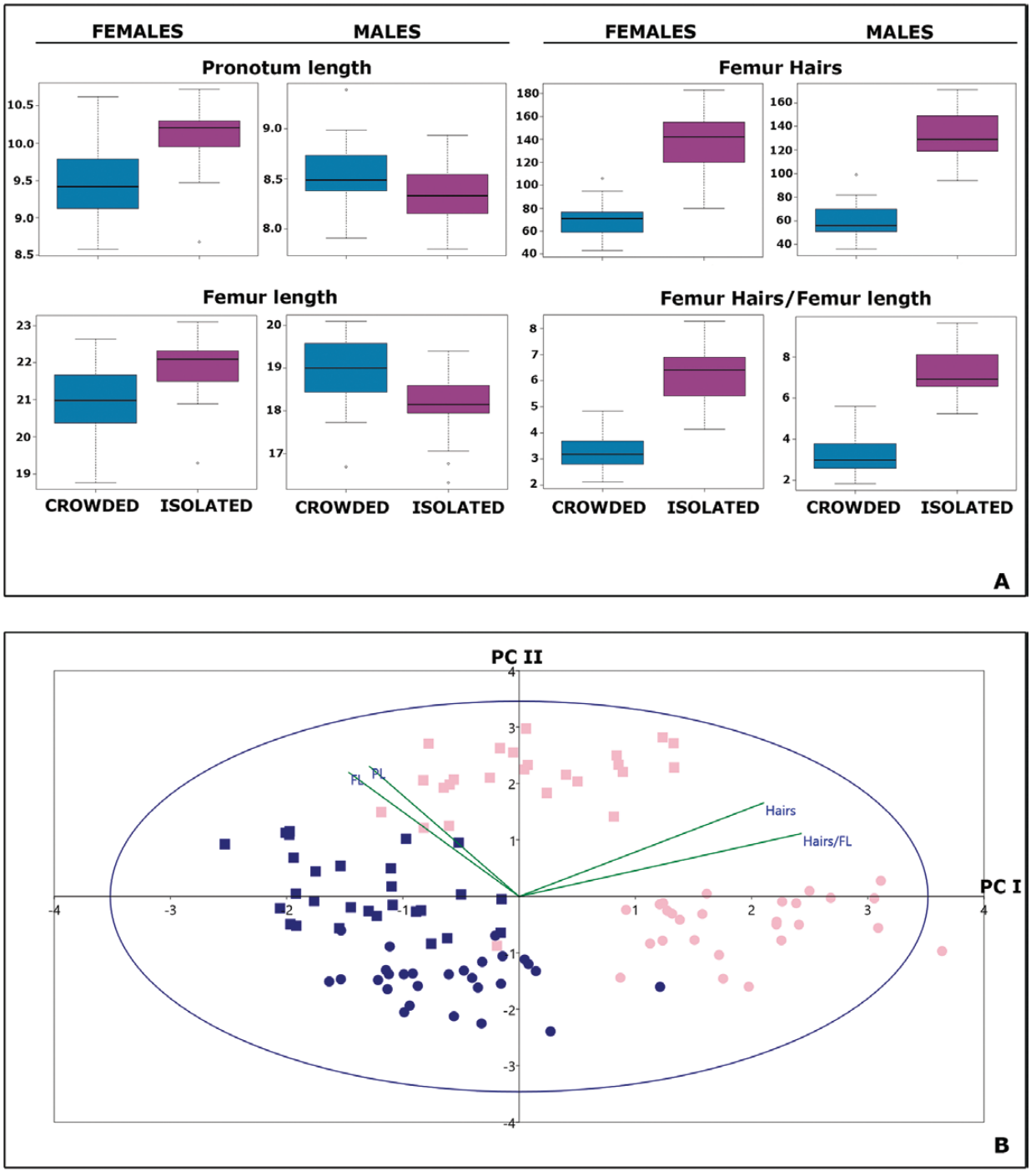


**Fig. 6.** (A) Density-dependent reaction norms in morphology (size, number of hairs on the hind femur, and ratio between the number of hairs and hind femur length) for female and male last instar nymphs of *Schistocerca cancellata*. Bars as indicated. (B) Biplot showing the results of PCA on the morphology (pronotum and hind femur lengths, number of hairs on hind femur, and hairs/femur length ratio) for *S. cancellata* male (circles) and female (squares) last instar nymphs, in isolated (light color symbols) and crowded (dark color symbols) conditions.

solitarization is a slower process (Roessingh and Simpson 1994; Roessingh et al. 1998; Simpson et al. 1999, 2001; Rogers et al. 2003). We do not yet have a time-course of behavioral gregarization or solitarization in *S. cancellata*, but we predict that a similar pattern would be observed given the similar behavioral responses in both species. Considering that both *S. gregaria* and *S. cancellata* are adapted to arid or semiarid regions, although they occupy divergent places in the phylogeny of *Schistocerca* (Song et al. 2017), it is conceivable that both species could have experienced similar selective pressures, which may have promoted the convergent evolution of density-dependent behavioral plasticity. Therefore, while many of the characteristics of the gregarization of *S. cancellata* and *S. gregaria* are similar, it is not surprising that there are some differences (Zhang et al. 2019).

Nymphal coloration is one of the most conspicuous phenotypic traits of locust phase polyphenism, in which solitarious nymphs are typically green while gregarious nymphs are brightly colored in combination of black, yellow, red, or orange (Stower 1959;

**Table 3.** Values from two-way ANOVA test showing differences in morphology between isolated and crowded treatments for last instar nymphs of *Schistocerca cancellata*

| | Morphology | | | | |
|---|---|---|---|---|---|
| | Source | Partial $\eta^2$ | df | *F* | *P*-value |
| Pronotum length | Density | 0.06 | 1 | 6.9 | 0.0097* |
| | Sex | 0.77 | 1 | 347.8 | <0.001* |
| | Density:sex | 0.22 | 1 | 28.8 | <0.001* |
| | Residuals | | 104 | | |
| Femur length | Density | 0.002 | 1 | 0.2 | 0.6763 |
| | Sex | 0.772 | 1 | 352.3 | <0.001* |
| | Density:sex | 0.217 | 1 | 28.8 | <0.001* |
| | Residuals | | 104 | | |
| Femur hairs | Density | 0.774 | 1 | 355.2 | <0.001* |
| | Sex | 0.031 | 1 | 3.3 | 0.0711 |
| | Density:sex | 0.003 | 1 | 0.3 | 0.5772 |
| | Residuals | | 104 | | |
| Hairs/femur length | Density | 0.767 | 1 | 342.07 | <0.001* |
| | Sex | 0.055 | 1 | 6.09 | 0.0152* |
| | Density:sex | 0.079 | 1 | 8.91 | 0.0035* |
| | Residuals | | 104 | | |

*Significant differences between the two density conditions.

**Table 4.** Mean values (minimum and maximum values) of five linear measurements (in mm) and E/F and F/C ratios for crowded and isolated adults (females and males) of *Schistocerca cancellata*

| | Morphology (size)—adults | | |
|---|---|---|---|
| | Parameters | Crowded (*N* = 111) | Isolated (*N* = 29) |
| Females | Body length (BL) | 67.2 (52.26–75.3) | 66.01 (60–70.8) |
| | Tegmen length (E) | 52.22 (35.62–60.4) | 53.56 (48.8–58) |
| | Femur length (F) | 27.81 (22.88–31.8) | 27.96 (25.5–30.7) |
| | Head width (C) | 7.2 (5.69–11.4) | 6.5 (6–7) |
| | Pronotum length (PL) | 10.53 (6.3–11.7) | 10.19 (8.7–11.3) |
| | E/F | 1.88 (1.26–2.08) | 1.92 (1.77–2.09) |
| | F/C | 3.87 (2.75–4.51) | 4.31 (3.90–4.66) |
| | | Crowded (*N* = 93) | Isolated (*N* = 36) |
| Males | Body length (BL) | 58.12 (47.52–65.39) | 52.72 (47.5–56.1) |
| | Tegmen length (E) | 45.07 (36.16–50.57) | 42.20 (38.7–45.5) |
| | Femur length (F) | 24.2 (20.5–27.9) | 22.41 (17–25.3) |
| | Head width (C) | 6.22 (5.25–8.3) | 5.24 (4.4–5.6) |
| | Pronotum length (PL) | 9.16 (6–10.88) | 8.17 (6.7–8.9) |
| | E/F | 1.87 (1.35–2.29) | 1.89 (1.68–2.34) |
| | F/C | 3.9 (3.05–4.57) | 4.28 (3.47–4.78) |

Uvarov 1966; Pener 1991; Tanaka 2000, 2006; Pener and Simpson 2009; Tanaka et al. 2012, 2016). Compared to the behavior, the proximate mechanisms of color change are less understood in general with an exception to the density-dependent development of black patterns, which has now been conclusively demonstrated to be affected by the neuropeptide corazonin (Tawfik et al. 1999; Tanaka 2000, 2006; Sugahara et al. 2015; Tanaka et al. 2016). The background coloration seems to be affected by not only the density, but also temperature, humidity, as well as substrate coloration (Pener and Simpson 2009, Tanaka et al. 2012, 2016).

Our results have shown that the amount of black pattern and background coloration are significantly different between crowded and isolated nymphs (Fig. 7). In natural conditions, the coloration in gregarious nymphs is brighter than what we can induce in the laboratory, but showing the same patterns to those observed herein, except for the occurrence of yellow bright nymphs in gregarious conditions that are also seen in the field (Pocco and Cigliano, personal observations).

The coloration of adults is quite similar between the two phases, although the pattern of stripes in pronotum is faintly evident in gregarious adults. Besides, immature adults in the gregarious phase show a reddish coloration during the cold season, turning to a general pale color with yellowish hindwings as they mature (Kohler 1979). Phase color polyphenism also exists in hatchlings. Transgenerational (both maternal and paternal) effects of population density have been shown to affect hatchling coloration (and other traits) (Islam et al. 1994a,b; Bouaïchi and Simpson 2003; Tanaka and Maeno 2006; Simpson and Miller 2007), and the presence of any such effects in *S. cancellata* is unknown and needs further study. In *L. migratoria* and *S. gregaria*, the hatchlings from the eggs laid by the isolated females are mostly light gray and light pale green, respectively, whereas those hatchlings from the eggs of the

**Table 5.** Values from two-way ANOVA test showing differences in morphology (size) between isolated and crowded treatments for adults of *Schistocerca cancellata*

| | Morphology (size)—adults | | | | |
|---|---|---|---|---|---|
| | Source | Partial $\eta^2$ | *F* | df | *P*-value |
| Body length | Density | 0.13 | 40.4 | 1 | <0.001* |
| | Sex | 0.64 | 465.6 | 1 | <0.001* |
| | Density:sex | 0.06 | 16.5 | 1 | <0.001* |
| | Residuals | | | 260 | |
| Tegmen length | Density | 0.02 | 6.1 | 1 | 0.0141* |
| | Sex | 0.65 | 481.2 | 1 | <0.001* |
| | Density:sex | 0.10 | 28.3 | 1 | <0.001* |
| | Residuals | | | 260 | |
| Femur length | Density | 0.06 | 15.8 | 1 | <0.001* |
| | Sex | 0.65 | 492.7 | 1 | <0.001* |
| | Density:sex | 0.08 | 22.2 | 1 | <0.001* |
| | Residuals | | | 265 | |
| Head width | Density | 0.50 | 256.1 | 1 | <0.001* |
| | Sex | 0.65 | 487.6 | 1 | <0.001* |
| | Density:sex | 0.04 | 10.8 | 1 | 0.0011* |
| | Residuals | | | 261 | |
| Pronotum length | Density | 0.22 | 73.3 | 1 | <0.001* |
| | Sex | 0.61 | 409.4 | 1 | <0.001* |
| | Density:sex | 0.04 | 11.7 | 1 | <0.001* |
| | Residuals | | | 261 | |
| E/F | Density | 0.011 | 2.9 | 1 | 0.0876 |
| | Sex | 0.005 | 1.4 | 1 | 0.2393 |
| | Density:sex | 0.001 | 0.1 | 1 | 0.7123 |
| | Residuals | | | 265 | |
| F/C | Density | 0.353 | 144.90 | 1 | <0.001* |
| | Sex | 0.000 | 0.00 | 1 | 0.994 |
| | Density:sex | 0.002 | 0.60 | 1 | 0.441 |
| | Residuals | | | 265 | |

*Significant differences between the two density conditions.

**Table 6.** Mean duration (days) of each nymphal instar and adult stage for isolated and crowded treatments when reared at a constant 30°C in the laboratory

| | Mean ± SD | | *Z* | *P*-value |
|---|---|---|---|---|
| | Isolated | Crowded | | |
| Instar I | 6.76 ± 1.33 | 5.4 ± 1.36 | −9.19 | <0.001* |
| Instar II | 5.8 ± 1.53 | 4.45 ± 1.11 | −8.59 | <0.001* |
| Instar III | 6.12 ± 1.37 | 4.63 ± 1.53 | −8.92 | <0.001* |
| Instar IV | 7.46 ± 2.32 | 5.05 ± 1.57 | −7.9 | <0.001* |
| Instar V | 7.96 ± 2.2 | 6.56 ± 1.61 | −4.76 | <0.001* |
| Instar VI | 13.82 ± 4.18 | 9.53 ± 1.58 | −6.23 | <0.001* |
| Adult | 86.6 ± 55.7 | 57.95 ± 17.8 | −3.95 | <0.001* |

Values from *Z*-test (*Z*) and *P*-values are indicated.
*Significant differences between the two density conditions.

crowded parents are much darker in *L. migratoria* and dark, partially or almost completely black in *S. gregaria* (Pener and Simpson 2009). In *S. cancellata*, we observed that the hatchlings from isolated and crowded females during the first hours are in general light green with or without darker areas; if the nymphs are maintained in the crowded condition, the black pattern becomes conspicuous, and if the nymphs are kept in isolation within the few hours after hatching, the greenish or light brown coloration is maintained, with or without darker areas. If the nymphs are placed in isolation after several hours of crowding after hatching, the changes to a greenish coloration are evident after the first molt. In *S. gregaria*, Tanaka and Maeno (2006) found that the color of the hatchlings did not change during the first stadium and the effect of rearing density on the color became evident only in the second stadium.

We report that the nymphal body size in *S. cancellata* is affected by rearing density and there is a sex-dependent pattern. Last instar female nymphs reared in isolation are larger than crowded ones, while isolated male nymphs are smaller than crowded males. A similar pattern was found in two nonswarming *Schistocerca* species (Gotham and Song 2013). Our results indicate that the difference in body size between males and females becomes less pronounced in crowded individuals than in isolated ones. This sex-dependent pattern persists in the adults as well. The size of the female adults does not differ much between the two density conditions, although the head is significantly wider and the pronotum slightly larger in crowded females than in isolated ones. On the contrary, there are clear size differences in males, in which crowded male adults are significantly larger than isolated males. This is an opposite pattern from what is known from other locust species. In *S. gregaria*, *L. migratoria*, and the red locust, *Nomadacris septemfasciata* (Serville, 1838) (Cyrtacanthacridinae), solitarious females are larger than gregarious ones while solitarious males are slightly smaller than gregarious ones (Uvarov 1966, Pener and Simpson 2009). However, in other locusts, such as *Dociostaurus maroccanus* (Thunberg, 1815) (Gomphocerinae), *Locustana pardalina* (Walker, 1870) (Oedipodinae), and *C. terminifera*, gregarious adults are larger than solitarious adults independently of the sex (Uvarov 1966, 1977; Pener and Simpson 2009).

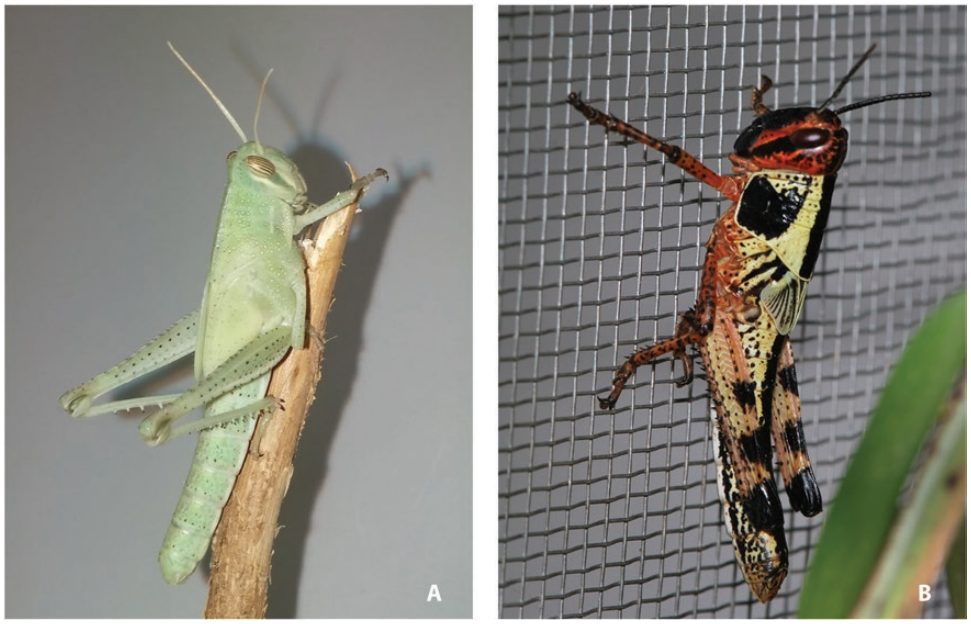


**Fig. 7.** *Schistocerca cancellata* nymphs. Last instar nymph under isolated conditions (A) and penultimate instar nymph under crowded conditions (B).

Differences between locust species are to be expected due to the independent evolution of phase characteristics (Zhang et al. 2019), and this is clearly demonstrated by *S. cancellata* in the classic measures of phase change, the E/F and F/C morphometric ratios. In *S. gregaria*, *L. migratoria*, *N. septemfasciata*, and *L. pardalina* the F/C ratio is lower in gregarious than in solitarious adults; although the E/F ratio is higher in gregarious locusts (Uvarov 1966, Nolte 1967, Pener and Simpson 2009). In *S. cancellata*, we found that the E/F ratio did not show differences between the density conditions regardless of sex, but the F/C ratio was significantly lower in crowded adults than in isolated ones. This indicates that the head width was wider in crowded adults than in isolated ones, which had been postulated to be related to the voracious feeding capability of the gregarious locusts (Uvarov 1966).

One of the most intriguing findings about the proximate mechanism of locust phase polyphenism is that the main morphological structure for detecting density is the series of mechanoreceptor hairs located on the outer face of hind legs in *S. gregaria* (Simpson et al. 2001). Mechanical stimulation of these hairs using a brush would cause the process of behavioral gregarization (Simpson et al. 2001), and we have strong evidence that the neurons from these mechanoreceptors are connected to thoracic ganglia where serotonergic neurons produce serotonin in response to increases in density (Anstey et al. 2009, Rogers and Ott 2015). Rogers et al. (2003) found that the isolated last instar nymphs of *S. gregaria* had about 30% more mechanosensory trichoid sensilla on the outer surface of the hind femur than the crowded nymphs, implying that the solitarious locusts are endowed with more sensory structures to detect the local population density. Our examination of the outer face of the hind femur in *S. cancellata* shows that isolated nymphs had about 50% more hairs than crowded nymphs. This finding indicates that *S. cancellata* may use the same body part as the main detector of density as *S. gregaria*. However, this may not be because the two species are in the same genus. In two nonswarming *Schistocerca* species, Gotham and Song (2013) found that crowded nymphs had more mechanoreceptor hairs than isolated ones, which is the opposite pattern. In the Australian plague locust, *C. terminifera*, the mechanoreceptors involved in detecting stimuli associated with population density are located on the antennae and not on the hind femur, which means that different species may differ in the proximate mechanisms of phase change. The fact that both *S. cancellata* and *S. gregaria* show the same pattern, while other nonswarming *Schistocerca* species show the opposite pattern (Gotham and Song 2013), indicates that this physiological and neurobiological mechanism could have evolved convergently in the two locust species.

The availability of the colony of *S. cancellata* has also provided a unique opportunity to generate baseline life history parameters. Interestingly, we find that the rearing density also affected the development time. The mean duration of nymphal development as well as the duration of adult stage were longer in the isolated than in the crowded conditions, similar to *S. gregaria* but the difference seems to be much greater in *S. cancellata*.

We found that there were six nymphal instars for both isolated and crowded *S. cancellata*, which is consistent with previous studies conducted in the laboratory (Barrera and Turk 1983, Sanchez et al. 1997). However, five instars were recorded in nature for gregarious *S. cancellata* (Daguerre 1953, Gastón 1969, Ministerio de Agricultura de la Nación 1946, COPR 1982). As it has been recorded for *S. gregaria* (Pener 1991), the number of eye stripes reflects the number of instars from hatching to adult in *S. cancellata*. Both isolated and crowded locusts have six nymphal instars and therefore the number of stripes in adults is seven. However, the crowded *S. gregaria* has five nymphal instars and the adult stage, and therefore, the number of eye stripes is always six, while the solitarious adults has an extra eye stripe due to an extra molt (Uvarov 1966, Pener 1991, Maeno and Tanaka 2009).

Data on locust density, resource abundance, and resource distribution patterns are fundamental in order to evaluate the risk of locust gregarization within a population (Sword et al. 2010). Additionally, a precise morphological characterization of phases is fundamental to improve the preventive strategies against locusts (Lecoq et al. 2011). From an operational perspective, the phase transformation threshold is crucial for an earlier prevention as shown by Lecoq et al. (2011) who developed a hopper pigmentation typology based on the results of a field study on the red locust (*N. septemfasciata*) and were able to detect

the first pigmentary sign of gregarization. The South American locust exhibits an extreme form of density-dependent phenotypic plasticity in nymphal behavior, coloration, size, and life history traits, as well as in adult size as demonstrated in this study. However, further detailed evaluation in the field of the traits registered herein would help to detect early signs of gregarization in *S. cancellata* and to evaluate the locust population prior to reach the phase transformation threshold.

## Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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