

Full Length Research Paper

Density-dependent phenotypic plasticity in body coloration and morphometry and its transgenerational changes in the migratory locust, *Locusta migratoria*

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Accepted 2 August, 2011

Migratory locust, *Locusta migratoria* (Orthoptera, Acrididae) changes phase in response to population density. By rearing nymphs from a solitarious (isolated-reared) and gregarious line at three different densities, we examined the effects of rearing density on body coloration and morphometry at the last nymphal instar, adult stage and hatchlings of the subsequent generation. Changes in density lead to phase transformation shown by a shift in the body coloration and size to either direction depending on population density. Nevertheless, the complete shift of solitarious locusts to gregarious phase cannot be acquired in the first generation of crowding and solitarious body coloration still appears even at high density (100 locusts/cage). In both phases, the shift of body colour in response to the variation of population density was more rapid than the morphometry. Adult rearing density affected also the progeny body colour and size. However, the parental prehistory and the environmental conditions of the offspring were also important to modify the phase characteristics. The most important gregarious characteristics of hatchlings (black body colour and large size) were observed if parents were maintained at the density of 40 per cage. These characteristics depend not only on parents phase state but also depend on the food abundance. We found a positive correlation between the darkness of body colour and size of hatchlings and this effect was more pronounced in gregarious line.

Key words: *Locusta migratoria*, rearing density, phase characteristics, progeny, parents' prehistory.

INTRODUCTION

The migratory locust, *Locusta migratoria*, and the desert locust, *Schistocerca gregaria*, both show density-dependent phase polyphenism, in which they transform reversibly between two extreme phases, solitary and gregarious, in response to population density (Chopard, 1938; Uvarov, 1966). This transformation involves graded changes in a number of characteristics, including behavior, colour, reproduction, development, morphometry and endocrine physiology (Albrecht, 1967; Dale and Tobe, 1990; Girardie, 1991; Pener, 1991). A series of intermediates (transient) exist between the two extreme

phases during a transient period from one extreme phase to another. Individuals in low-density populations (the solitary phase) are characterized by a uniformly coloured body small eggs and hatchlings size, a relatively small ratio of fore-wing length to hind femur length and sedentary and solitary behaviors. Individuals in high-density populations (the gregarious phase) have a dark body colour, large eggs and hatchlings size, a large ratio of fore-wing length to hind femur length, and migratory and gregarious behaviours (Ben Hamouda et al., 2009; Dale and Tobe, 1990; Faure, 1932; Pener, 1991; Pener and Yerushalmi, 1998; Tanaka, 2006; Uvarov, 1921, 1966). Longer-term crowding of locusts results not only in increased behavioral tendencies for aggregation, but also in a change of morphology, both of which are

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under hormonal control (Kraus and Ruxton, 2002). Maternal effects are sometimes interpreted as an important mechanism that allows a (presumably adaptive) phenotypic change in offspring depending upon the environmental cue perceived by the parents. In other words, female parents might adjust the phenotypes of their offspring in response to certain cues that predict the environment her offspring will encounter, in a way that enhances offspring fitness (Bernardo, 1996). One of the most important features of phase changes in locusts is the fact that offspring exhibit some of the phase characteristics of their mother. Hence, phase change is a cumulative process that is transmitted from one generation to the next (Albrecht et al., 1959; Faure, 1932; Papillon, 1960; Tanaka and Maeno, 2008; Uvarov, 1966). Such carry-over from one generation to the next of phase characteristics such as body colour, morphometry, body weight and mature oocyte numbers has been amply documented in the literature (Albrecht et al., 1959; Gunn and Hunter-Jones, 1952; Hunter-Jones, 1958; Papillon, 1970). The mechanism(s) of trans-generational transfer of behavioural phase state has been studied (Bouaïchi et al., 1995; Hägele and Simpson, 2000; Islam et al., 1994a, b; McCaffery et al., 1998; Simpson et al., 1999). Variation in these traits is continuous and not discrete, and trans-generational steps proceed toward the gregarious phase for several generations (Uvarov, 1966). Under both in the field and laboratory, crowding induces characteristics of the gregarious phase, whereas isolation promotes those of the solitary phase. However, phase transformation is a complex phenomenon. Some phase characteristics change within hours, whereas others take longer and show changes in the next stadium, or even in the next generation (Pener, 1991; Uvarov, 1966, 1977).

Most studies concerning the effects of density on phase characteristics and their transgenerational changes have been carried out in *S. gregaria*. Therefore, in the present study, the effects of population density on phase characteristics in last instar nymphs, adults and their progeny were examined in *L. migratoria*.

MATERIALS AND METHODS

Insects

Experiments were carried out with solitary and gregarious colony of *L. migratoria*. This strain was maintained for more than 6 years at the High Institute of Agronomy of Chott-Mariem, Tunisia. The colony was brought to the National Institute of Agrobiological Sciences at Ohwashi, since the summer of 2009 by permission from the Yokohama Plant Protection Station. Preliminary analysis of mitochondria DNA suggests that this strain belongs to the southern clade of this species that includes strains from Africa, France, Australia, Timor, Southern China and Southern Islands of Japan, and is distinctly different from the northern clade that includes strains of most areas of Japan and China (Tokuda and Tanaka, unpublished data). The crowd-reared colony was maintained at 30°C in large wood-framed cages (42 cm × 22 cm × 42 cm; 0.038 m³). Each cage was covered with nylon screen mesh except for the wood floor and the front sliding door, which was composed of a transparent acrylic plate. Locusts were fed cut grass inserted in

water jars and wheat bran. Grass was changed every 1 or 2 days. *Bromus* grass was grown in crop fields by the Field Management Department of the National Institute of Agrobiological Sciences at Ohwashi. Locusts lay egg pods in moist sand held in plastic cups (volume of 380 ml) which were incubated at 30°C.

Rearing methods

To examine the effect of rearing density on phase characteristics, hatchlings obtained from both crowd-reared and isolated-reared colonies were reared at densities of 20, 40 and 100 individuals per cage in a well-ventilated room (30°C) until their adult stage. Egg pods collected from adults were incubated at 30°C. Egg length was measured with an ocular micrometer in a binocular microscope and hatchlings were checked for their body weight and colour.

Scoring of locust body colour

To quantify the density effects on body colouration, locusts were photographed using a scanner (Epson GT-X770, Japan) connected to a computer using commercial software, Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA). Locusts were chilled on ice for 15 min and placed with one side down on the glass table of the scanner for photographing. The image type used was 48-bit colour at a resolution of 1200 d.p.i. for hatchlings and 600 d.p.i. for last instar nymphs and adults. Hatchling body colour was observed 6 to 12 h after hatching. Hatchlings were divided into three colour groups based on the method of Hamouda et al. (2009): whitish (W), grey (G) and black (B). The last instar nymphs body colour was categorized by five colours: four graded of beige body colour according to the darkness and without or with black patterns, respectively (B1) and (B2, B3 and B4) and green body colour (G). Adults were subdivided to five colours: beige without black patterns (B1), beige with black patterns (B2), black (BL), grey (G) and yellow (Y). Statistical differences were analysed by multiple comparison using Student–Newman–Keuls test ($p \leq 0.05$) (SPSS.10 software).

Measurements of morphometric ratios

Electronic sliding callipers (accuracy 0.01 mm) were used to measure the following classical morphometric phase characteristics for last instars nymphs and adults in order to determine E/F, F/C and H/P ratios: E = length of forewing, F = length of hind femur, C = maximum head width, H = maximal height of pronotum and P = length of pronotum, (Joly, 1968; Pener and Yerushalmi, 1998; Uvarov, 1966). The statistical analysis was carried out using a multiple comparison using Student–Newman–Keuls test ($p < 0.05$) and discriminant analysis (SPSS.10 software).

RESULTS

Effects of different rearing densities on body colouration at the last nymphal instar and the adult stage in nymphs from gregarious and solitary lines

Figure 1 shows that rearing gregarious hatchlings in groups of 20 led to the appearance of solitary body colouration (B1) in 11% of the last instar nymphs (8% in the case of females (A) and 3% for males (B)). No solitary body colouration was observed at a density of 40 or

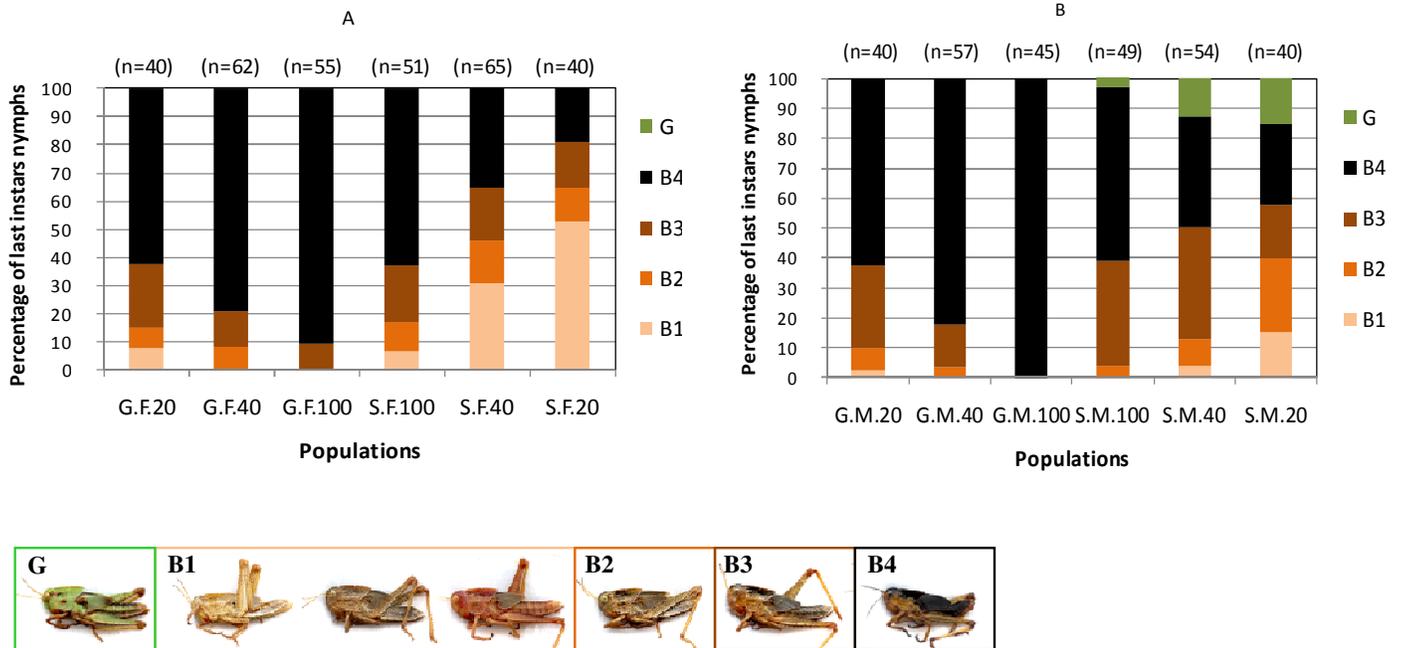


Figure 1. Effects of nymphal rearing density on body colouration at the last nymphal instar in *L. migratoria* individuals from a gregarious and solitary line. (A) G.F/S.F: gregarious/solitary females; (B) G.M/S.M: gregarious/solitary males; 20, 40 and 100: the number of locusts/cage. Numbers in parentheses (n) indicate the number of individuals.

100 gregarious nymphs /cage. The intermediate body colouration (B2 and/or B3) appeared in different proportions at the three densities of gregarious females (Figure 1A). However, half of the total proportion of this colouration was provided in G.F.20. No intermediate body colouration was observed at the density of 100 gregarious males per cage (Figure 1B). Gregarious body colouration (B4) was observed at the three densities in nymphs from gregarious lines depending on population densities.

Figure 1 shows that even under crowded conditions, nymphs from solitary line conserved their solitary body colouration (B1 and/or G) at the last stage. The highest proportion of solitary body colouration (53% in the case of females (A) and 30% for males (B)) was observed at a density of 20 nymphs. At high density (100 individuals /cage), only 7 and 4% of individuals developed solitary body colouration for females and males, respectively. Intermediate body colouration was distributed almost equally at the three densities and the highest proportion of gregarious body colouration was found at a density of 100 nymphs /cage.

Figure 2A shows that in female adults, the darkness of body colour was correlated positively to an increase in the number of individuals per cage. In females from solitary lines, green colour did not appear when the locusts were reared in a group of 100. In males (Figure 2B), yellow colour, typical of gregarious phase, was observed at different densities in differential proportions. The

highest proportion of males with yellow body colour (75%) was obtained at a density of 40 locusts per cage. In adults from solitary lines (Figure 2B), a density of 100 produced the highest proportion of yellow males (74%), but also yielded individuals with green colour (8%).

Effects of different rearing densities on morphometric ratios at the last nymphal instar and the adult stage in nymphs from gregarious and solitary lines

The variation in rearing density did not affect the length of femur (F) and the height of pronotum (H) of nymphs from gregarious line (Figure 3). In nymphs from solitary line, rearing density resulted in significant differences in F and H; they increased considerably when the population density decreased, although this was observed only in females (Figure 3A and C). In the case of males, we found that two groups were statistically different by the height of pronotum; the first group of gregarious nymphs was characterized by a low H and the second contains solitary nymphs with high H (Figure 3D).

Discriminant analysis was performed on different population densities for E/F, F/C and H/P ratios. In the case of females (Figure 4A), the first discriminant factor exhibited 90.8% of the total variability. It separates between two groups; the first includes gregarious females while solitary ones were contained in the second group.

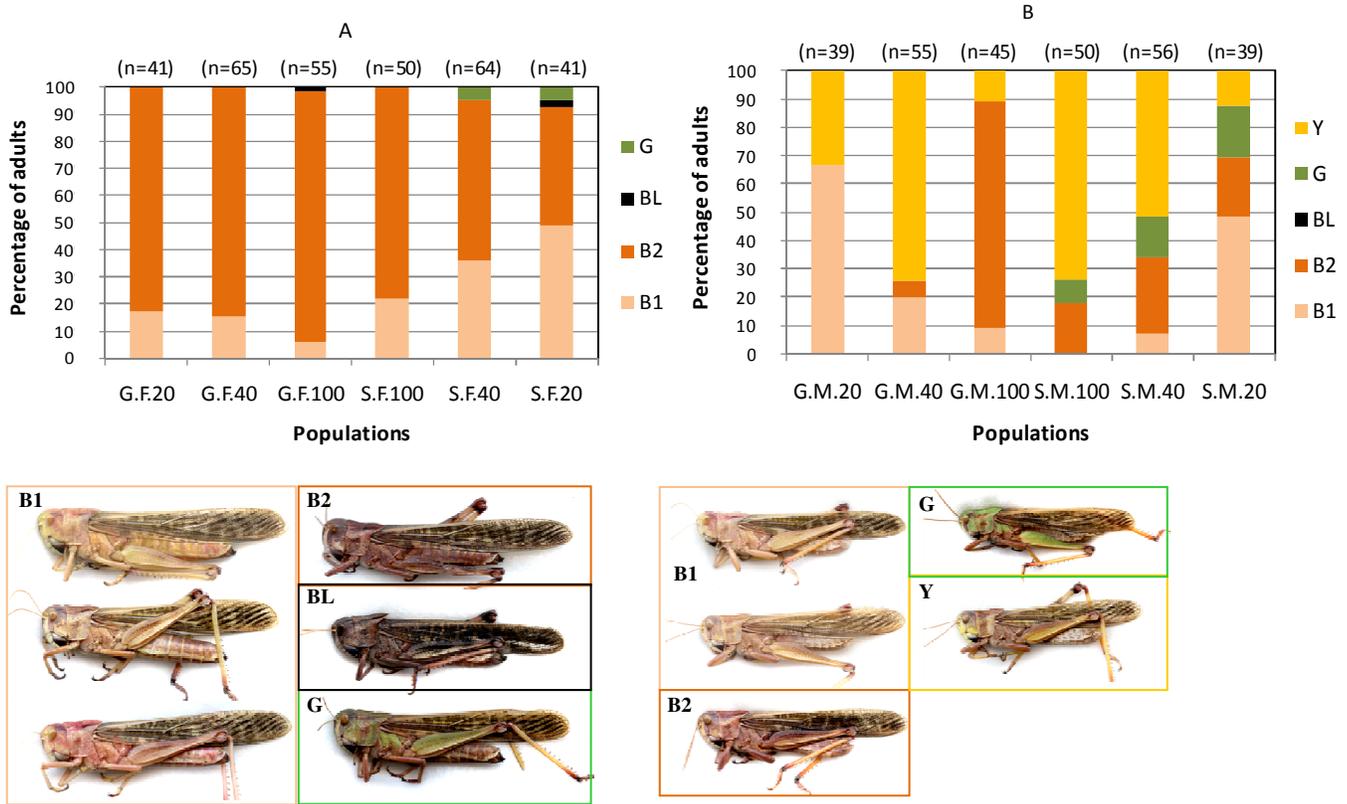


Figure 2. Effects of nymphal rearing density on body colouration at the adult stage in *L. migratoria* individuals from a gregarious and solitary line. (A) G.F/S.F: gregarious/solitary females; (B) G.M/S.M: gregarious/solitary males; 20, 40 and 100: the number of locusts/cage. Numbers in parentheses (n) indicate the number of individuals.

This factor was correlated negatively to the F/C ratio, on the side of the solitary groups. This means that solitary females have the highest F/C ratio. The same factor was positively correlated with E/F ratio towards gregarious females. In the case of males (Figure 4B), the first discriminant factor explains 90% of the total variation of the complete data sets. It was related positively to F/C on the side of solitary groups and negatively to E/F on the side of gregarious males. It appears that morphometrical segregation was established according to the origin of populations studied (gregarious or solitary phase).

Effects of parental rearing density on hatchlings phase characteristics

Egg length was compared among eggs laid by gregarious female adults maintained at different rearing densities (Figure 5). The mean egg length at a density of 20 locusts /cage was 5.71±0.4 mm; (n = 595), which was significantly shorter than those produced at a density of 100 (5.85±0.3 mm; n = 437) and 40 locusts /cage (6.12±0.4 mm; n = 442). Females which were derived from solitary line and reared at a density of 40 locusts

/cage produced the smallest eggs (5.17±0.3 mm; n = 492).

Concerning the body weight of hatchlings, Figure 6 shows that the density of 40 gregarious locusts /cage yielded the largest hatchlings (1.51±0.2; n = 139) in comparison with the density of 20 (1.32±0.2; n = 122) that showed the smallest hatchlings and the density of 100 (1.39±0.2; n = 155). It seems that the density of 40 locusts /cage was the best to induce large hatchlings. The difference was not significant between solitary locusts maintained at 20 and 40 locusts /cage which had 1.19±0.2; n = 95 and 1.21±0.1; n = 165 respectively. The smallest hatchlings were obtained from a density of 100 (1.14±0.1; n=113) but statistically, the difference was not significant.

In the same purpose, it seems that the density of 40 individuals/cage induces the highest proportion of black hatchlings (Figure 7). For both gregarious and solitary phase, there was a positive correlation between hatchlings body weight and their body colouration except for hatchlings from solitary females maintained at 100 (Table 1).

No correlation was found between eggs length and mother's age at deposition except in gregarious females

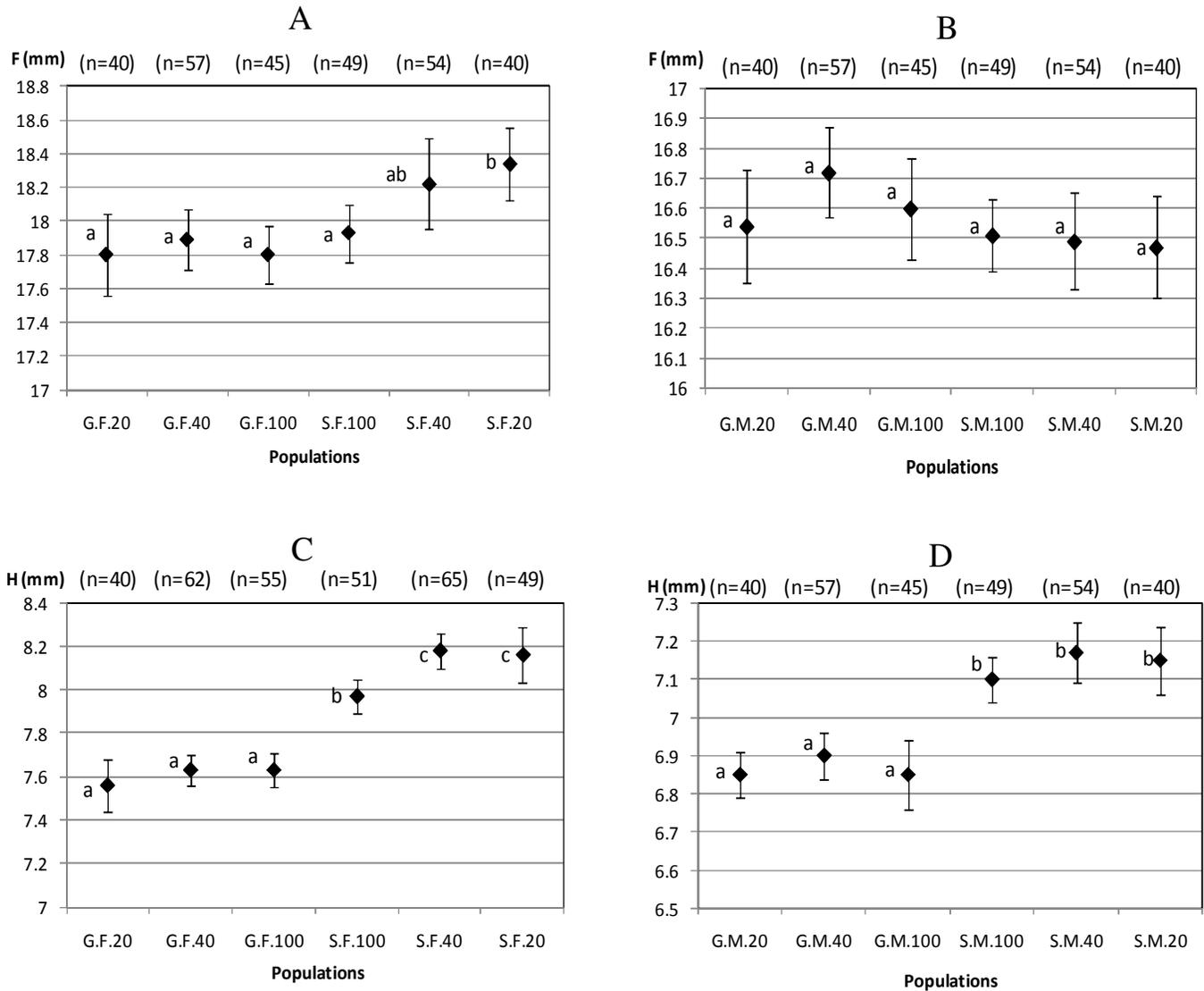


Figure 3. Effect of crowding density of hatchlings on gregarious and solitary last instar nymphs morphometry. (A): F for females; (B): F for males; (C): H for females; (D): H for males; G.F/S.F: gregarious/solitary females; G.M/S.M: gregarious/solitary males; H: maximal height of pronotum; F: length of hind femur. Numbers in parentheses (n) indicate the number of individuals. Different letters in each panel indicate a significant difference among H and F values at $p \leq 0.05$ by Student–Newman–Kuels test.

maintained at the densities of 20 ($r = -0.438$; $p \leq 0.05$) and 40 ($r = -0.171$; $p \leq 0.05$) that tended to produce smaller eggs as the female adults grew older (Figure 8). Figure 9A shows that gregarious females maintained at low density (20 individuals/cage) produce small hatchlings with age ($r = -0.263$; $p \leq 0.05$). While gregarious females maintained at 100 individuals/cage and solitary females maintained at 40 locusts/cage (Figure 9B) tend to produce bigger hatchlings, respectively ($r = 0.225$; $r = 0.361$; $p \leq 0.05$).

DISCUSSION

In the present study, we examined the effect of

population density by rearing gregarious and solitary hatchlings at three conditions: 20, 40 and 100 individuals /cage. The target of this study was to establish a system by which we could understand the relationship between the population density, phase changes and the trans-generational changes of phase characteristics in the migratory locust, *L. migratoria*. Our experiments confirmed the finding that the shift of various characteristics from one phase to another was density dependant and this effect was transgenerational in both phases. However, most studies concerning this phenomenon were done on *S. gregaria*. Consequently, we are interested in analyzing more of the subsequent occurrence on *L. migratoria*.

As shown previously by Hamouda et al. (2009) and

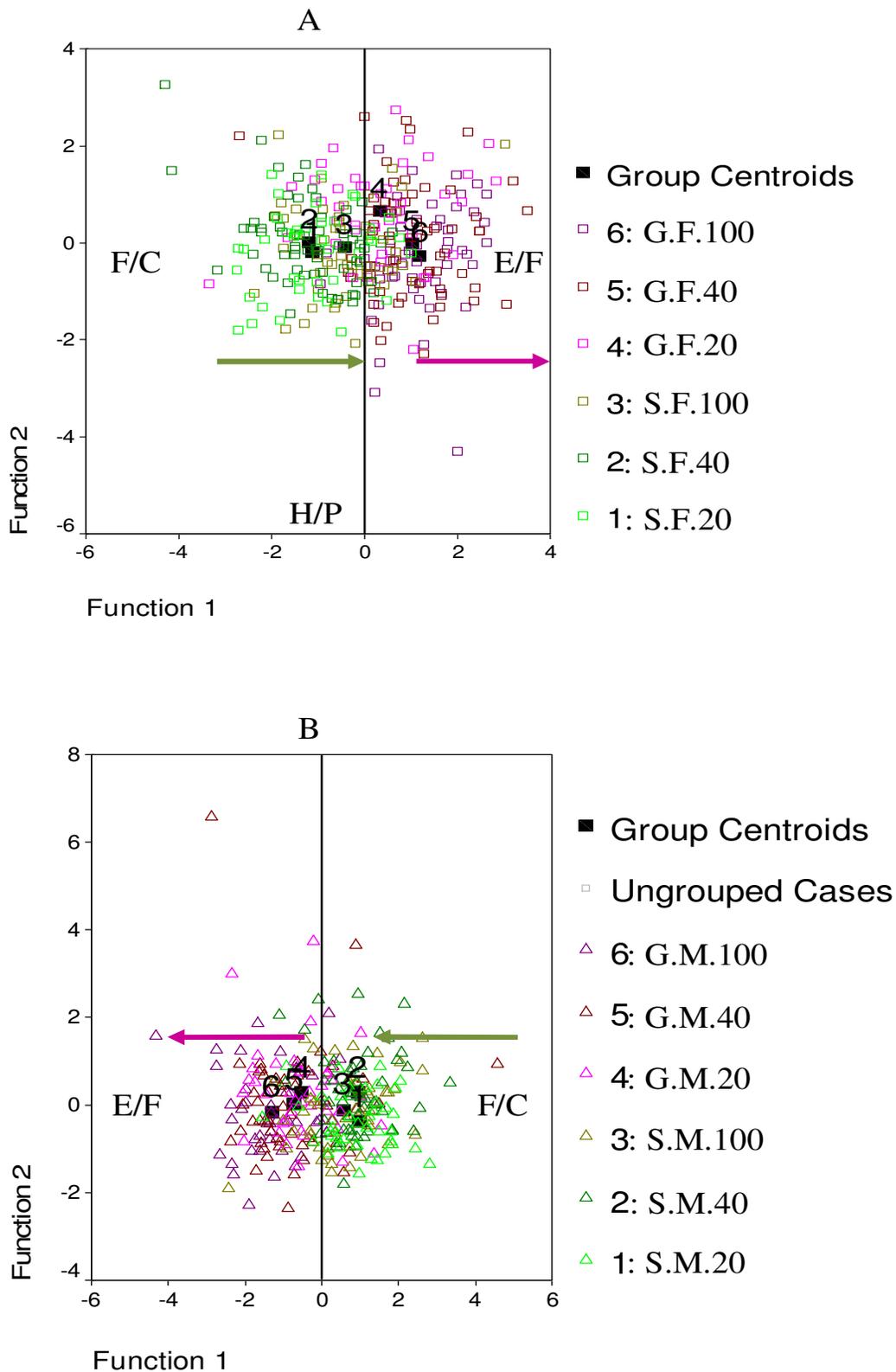


Figure 4. Main variables distribution measured in response to different population densities of females (A) and males (B) harvested in the factorial plan defined by the discriminant analysis. G.F/S.F: gregarious/solitary females; G.M/S.M: gregarious/solitary males. 20, 40 and 100: the number of locusts/cage. Arrows show the direction of the evolution of gregarious and solitary populations in response to the increase of the number of locusts/cage.

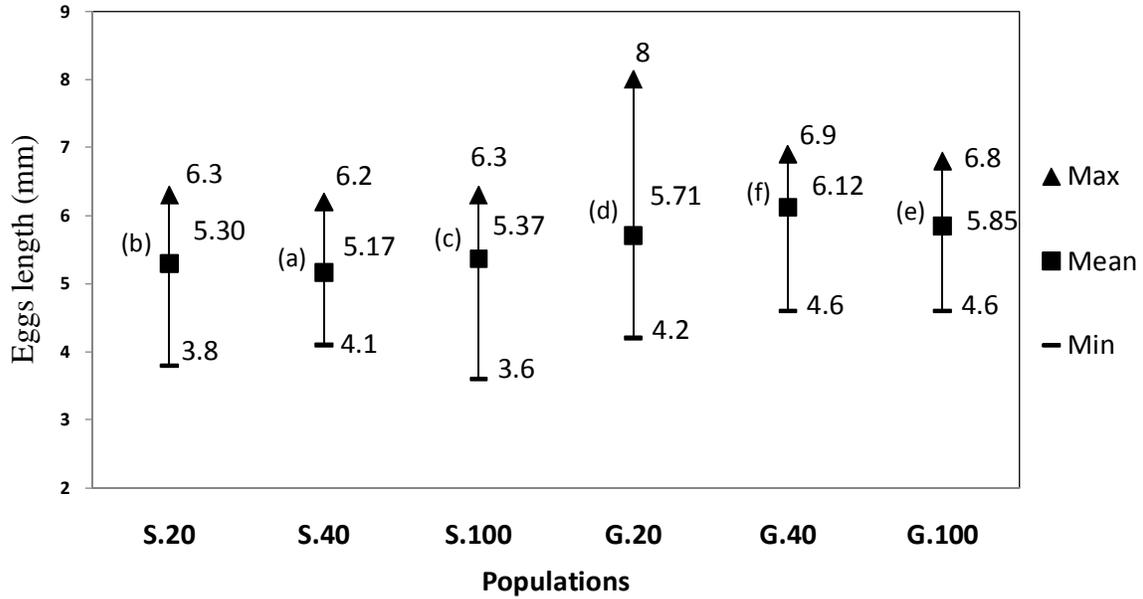


Figure 5. Effect of parental densities on solitary and gregarious eggs length. Different letters in each panel indicate a significant difference among eggs length at $p \leq 0.05$ by Student–Newman–Kuels test.

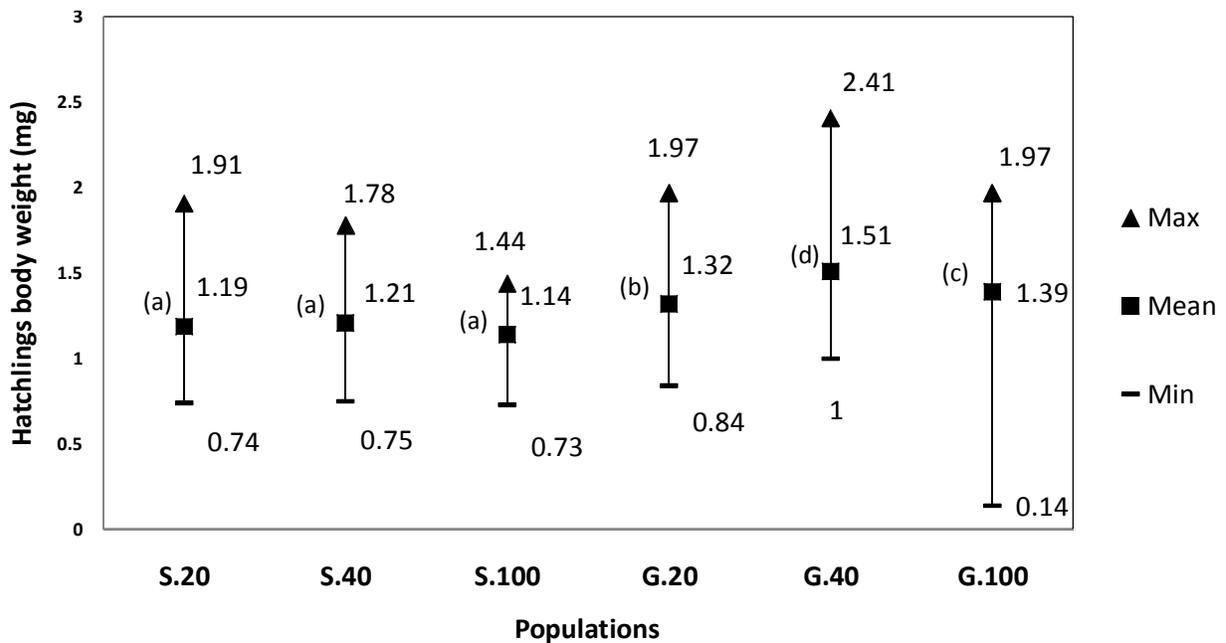


Figure 6. Effect of parental densities on solitary and gregarious hatchlings body weight. Different letters in each panel indicate a significant difference among eggs length at $p \leq 0.05$ by Student–Newman–Kuels test.

Gullan and Cranston (2010) by splitting a single locust egg pod into two: rearing the offspring at low densities induces solitary locusts, whereas their siblings reared under crowded conditions develop into gregarious locusts. The body colouration was one of the most rapid phase polyphenism characters that changes depending on the population density, and the development of black

pigmentation in response to change in density is widespread in grasshoppers (Applebaum and Heifetz, 1999, Pener, 1991, Rowell, 1967, 1971).

In the present experiments, solitary nymphs that were whitish at the early stages tended to exhibit an orange body colour with black patterns in last nymphs stadium. Indeed, at a density of 20 locusts /cage, 20% of females

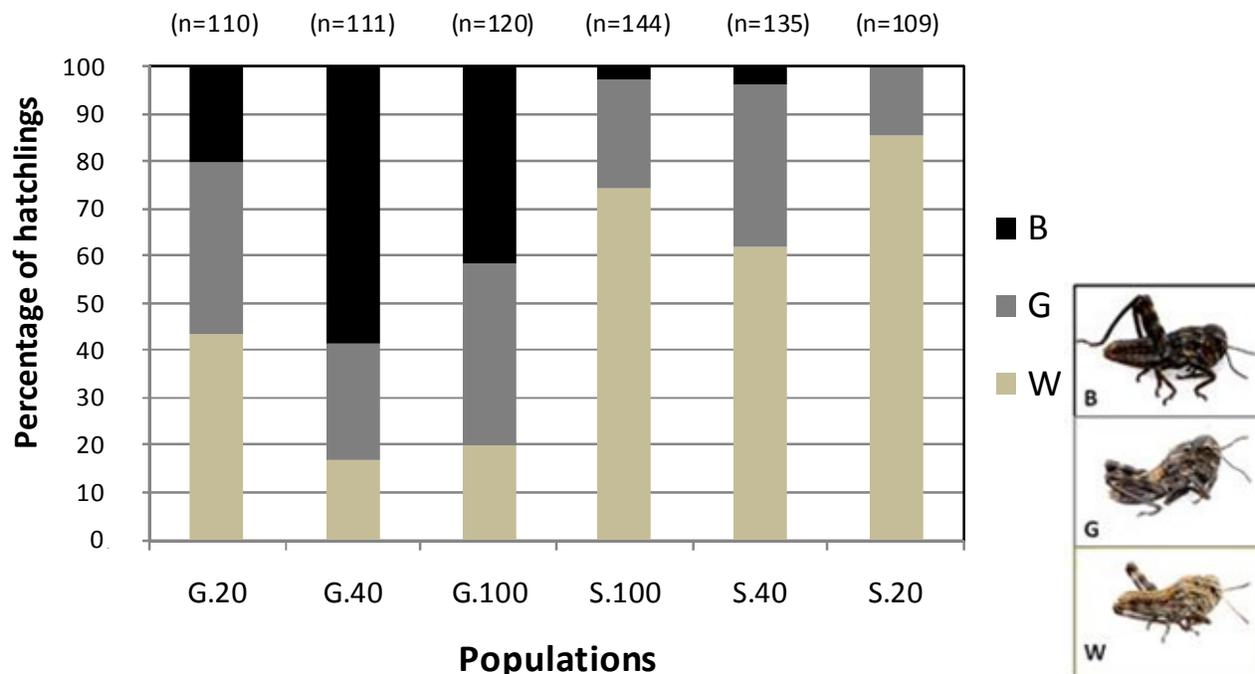


Figure 7. Effect of parental densities on solitary and gregarious hatchlings body colour. B: black; G: grey; W: whitish body colour of hatchlings. Numbers in parentheses (n) indicate the number of hatchlings.

Table 1. Matrix structure of F/C, E/F, and H/P ratios in discriminant analysis for gregarious and solitary populations maintained at different densities for each sex.

Morphometrical ratio		Discriminant factors	
		1	2
Females	F/C	-0.885*	0.071
	E/F	0.783*	0.579
	H/P	0.349	-0.716*
Males	F/C	0.958*	0.147
	E/F	-0.631*	0.464
	H/P	-0.238	0.517

* Largest absolute correlation between each morphometrical ratios and any discriminant factor.

and 28% of males shifted their body colour from solitary to the gregarious phase. From these observations, we concluded that in laboratory conditions, a density of 20 solitary nymphs per a volume of 0.038 m³, in other words, 526 solitary nymphs per 1 m³ were sufficient to induce the gregarious body colouration in some nymphs since the first generation of crowding.

The response of body colouration to the increase of population density was observed on other species of locusts; in the desert locust, *S. gregaria*, a wide spectrum of body-colour variation has been found depending on the population density (Gunn and Hunter-Jones, 1952; Hunter-Jones, 1958; Maeno and Tanaka, 2006; Stower, 1959; Tanaka and Maeno, 2010). Duck (1944) found that

solitary larvae of *Schistocerca obscura* were green, but when reared two or more per cage were brown with more or less dense black markings. Larvae of *Spodoptera littoralis* which were reared in crowded condition produced darker coloured larvae and when they were reared in isolation they were pale in colour (Hodjat, 1970). 7 and 4% of solitary body colour, in females and males respectively, were observed when solitary nymphs were maintained at the density of 100. This result indicates that one generation of crowding solitary nymphs at high density (100 nymphs/ cage) was insufficient to the complete shift to the gregarious body colouration and the sensitivity of solitary nymphs to crowding was variable among individuals.

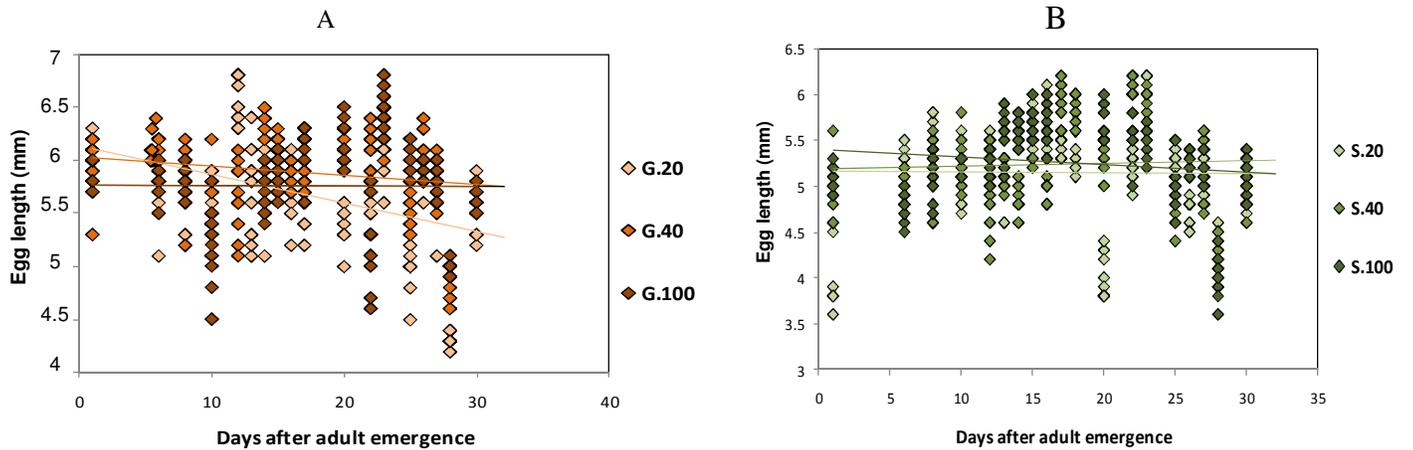


Figure 8. Temporal variation in eggs length of gregarious (A) and solitary (B) females under the variation of population density.

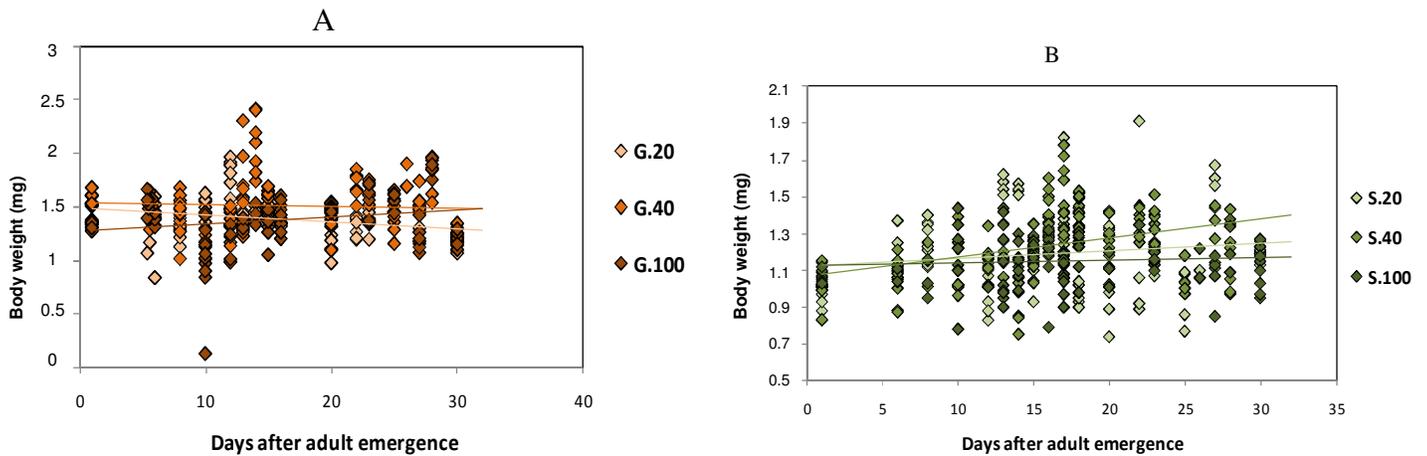


Figure 9. Temporal variation in hatchlings body weight of gregarious (A) and solitary (B) females under the variation of population density.

Rearing gregarious hatchlings at a low density (20 individuals /cage) resulted in the appearance of some individuals (11%) with solitary body colouration at the last nymphal instar. It means that maintaining a number of 20 gregarious nymphs per cage induces the solitariness of some nymphs but not the whole number. Furthermore, the shift to the solitary colouration was observed since the second nymphal stadium. We suppose that the body colour was influenced by the environmental conditions experienced during the previous stadium. This was in concordance with notes claimed by Cockrell (1933) in which he claimed that body colour seems to be inheritance when the first stage larva from *gregaria* would show the dark colouration. But this was due to the "locustine" in the egg, and disappeared in the second stage if the insect was reared alone. This it was observed also in *S.gregaria* (Hunter-Jones, 1958; Injeyan et al., 1979; Pener, 1991; Pener and Simpson, 2009; Uvarov, 1966).

At 40 gregarious nymphs/cage, solitary colouration

disappears completely. These results mean that the shift from gregarious phase to the solitary one starts when the density of gregarious nymphs decreases less than 20 into a volume of 0.038 m³. On other hand, rearing at high density (100 individuals/cage) consistently produced high proportion of gregarious body colouration in the late nymphal stage. These results were consistent with previous studies related to the body colour polyphenism expressed in response to the population density (Faure, 1932; Pener, 1991; Stower, 1959; Uvarov, 1966) and with studies made by Maeno and Tanaka (2008) on *S. gregaria*.

In solitary *L. migratoria* adults females, the proportion of gregarious body colouration (B2+BL) increased by 32% and the proportion of yellow (Y) males by 61% when the density of nymphs increased from 20 to 100 solitary individuals/cage. At a density of 100 individuals /cage, the green body colouration did not exceed 8% in solitary males, while it was 18% when the locusts were reared at a density of 20 individuals /cage. It was clearly suggested that the body colouration was positively correlated to the

rearing density and the phase state of locusts cannot still stable when individuals were exposed to the variation in density.

At a density of 20 solitary individuals /cage, and if we start by a 100% solitary body colouration at hatchling stage, 54% of solitary colouration in females and 67% in the case of males, remain at the adult stage. At 100 locusts, 22% of solitary colour persists in females and only 8% in the case of males. It seems that at low density, the response of body colouration of solitary adults to crowding was more rapid in the case of females than males. Contrariwise, at high density, the response of males was more pronounced.

Morphometric characteristics seem less labile than the body colouration to the variation of the population density and no significant changes were observed for H and F parameters in gregarious nymphs at all rearing densities tested. This might be because the individuals already had reached a gregarious phase by virtue of being reared under crowded conditions for many generations prior to the start of distributed them into different densities. We concluded that one generation of rearing gregarious nymphs at a density of 20 per cage was insufficient to allow them to change their morphometry to the solitary phase.

In the case of solitary nymphs, a significant difference was observed at a density of 100 females/cage; their F and H lengths were decreased significantly showing the shift from solitary to the gregarious phase when they were maintained at the density of 100. On the contrary, males could not change their morphometrical state since the first generation of crowding even at very high rearing density. It was clear that solitary females were more sensitive to crowding than males.

Discriminant analysis of morphometrical ratios of adults showed that, mainly, the switch of body size depends essentially on population densities; there was a clear discrimination between the gregarious and the solitary populations. Inside each group, the segregation was performed depending on the population density; in solitary females and males, as the number of locusts per cage increases, as the solitary populations brought closer to the gregarious locusts and their F/C ratio decreases. In the case of gregarious populations, as the number of individuals per cage increases, they rolled away from solitary populations and their E/F ratio increases. It has been proved that adult density influences the progeny characteristics in locusts (Hunter-Jones, 1958; Injeyan and Tobe, 1981; Maeno and Tanaka, 2008; Norris, 1950, 1952; Pener, 1991; Uvarov, 1966). However, most experiments were carried out on *S. gregaria*. What is the relationship between the prehistory of parents and the impact of rearing density on progeny? The present study demonstrated that maternal crowding influences progeny size in *L. migratoria* and the mother's rearing conditions shifts the phase state of her hatchlings accordingly: gregarious females tend to produce smaller

eggs and hatchlings and high proportion of whitish hatchlings at low rearing density (G.20). But at high density, food competition as one of the rearing conditions parameters may control the progeny size. This was observed at a density of 100 gregarious individuals /cage since the eggs and hatchlings size becomes smaller and the proportion of black hatchlings reduced by 16.89% from the density of 40 to the density of 100 individuals /cage. It seems that the suitable density of gregarious locusts to produce big eggs and large and black hatchlings was that of 40 individuals /cage. This means that the transmission of gregarious phase characteristics to the progeny in gregarious locusts was influenced not only by the population density, but also by the characteristics of rearing conditions, especially the food abundance.

Solitary females reared at a high density (100 individuals /cage) tend to produce larger eggs (5.37 mm) than the two others solitary lines but these eggs were smaller than the gregarious ones which among them the smallest mean was 5.71 mm. From these solitary eggs hatched small and whitish hatchlings. It seems that at high population density even they tended to produce larger eggs, solitary mothers yield small and whitish hatchlings. It was clear that in solitary phase, the phase characteristics of the progeny depends more to the prehistory and the phase state of parents than the population density. We concluded from this that solitary locusts are very sensitive to crowded conditions and food abundance appears to be important in the determination of eggs and hatchlings size and colour. This parameter may represent a limiting factor to produce large-sized hatchlings. Consequently, rearing density may play a role in the induction of the enlargement or the miniaturization of eggs and hatchlings indirectly by affecting their parents' size. In fact, it has been hypothesized by Tanaka and Maeno (2010) that crowding stimulus perceived is likely to be transmitted to the brain which in turn may cause some factor controlling egg size to be released. One possibility is that this factor is released in response to high adult density and acts on the ovary to increase egg size. Alternatively, it is released in response to low adult density and stimulates the ovary to reduce egg size. It has been shown also that adult females of *Locusta* from larvae reared in isolation weighed 1.5 g while others from larvae reared in crowds weighed only 1.2 g (Chapman, 1998). These findings indicate clearly that size of the parents may be influenced by rearing density in which their eggs and hatchlings size depend.

As shown in Table 1, there was a strong positive correlation between the darkness of body colour and size of hatchlings produced by gregarious line. The same conclusion was founded previously in the case of *S. gregaria* by Maeno and Tanaka (2009). Additionally, set apart the effect of phase, the larger the hatchling body size the larger the degree of darkening and this correlation is consistently observed in the case of gregarious as that of solitary phase. We found in 729 gregarious and

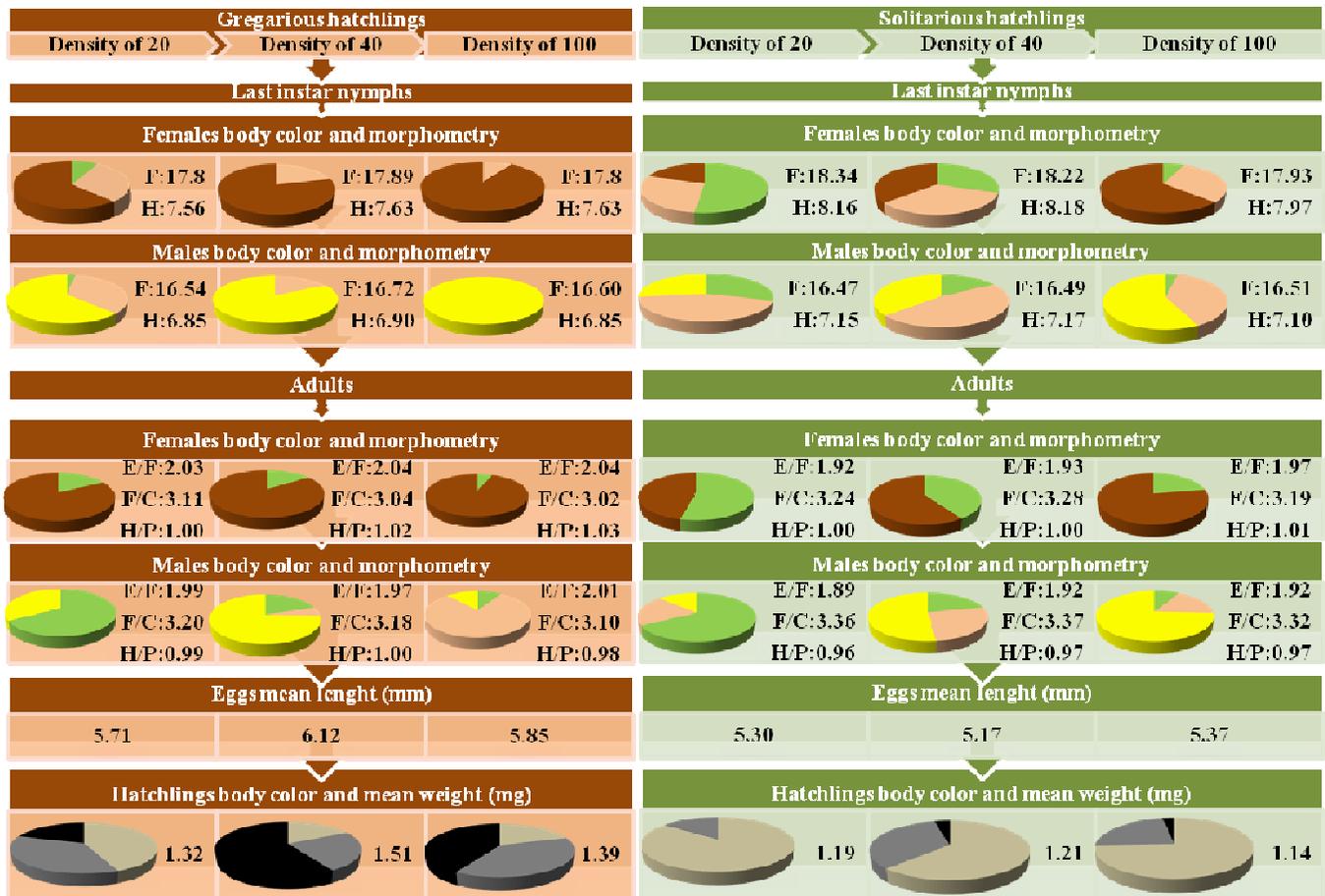


Figure 10. A synopsis showing the effect of the variation of population density on body color and morphometry of last instar nymphs, adults and their progeny. F: length of hind femur; H: maximal height of pronotum; E/F, F/C, H/P: morphometrical ratios of adults; : solitarious body color; : transient body color; : females gregarious body color; : males gregarious body color; : whitish, : grey and : black body color of hatchlings.

solitary hatchlings observed, significant differences in body weight between whitish, grey and black hatchlings. Among those hatchlings, the mean average of black hatchling (1.58 mg) was 1.35 times bigger than the mean average of whitish hatchling (1.17 mg). Whereas, grey hatchlings have a medium mean average of body size (1.34 mg). These observations were in concordance with those of Tanaka and Maeno (2008) in the case of *S. gregaria*; they found also that the correlation was observed even within the same egg pod that produces a mixture of green and black hatchlings.

Our results are very important for the study of locust phase polyphenism, especially the migratory locust, *L. migratoria*. In fact, a change of transgenerational of some phase characteristics in response to population density will help us to predict locust outbreaks. Figure 10 shows clearly the response of each stage to the variation of population density and the process of the transgenerational of the changes to the progeny.

ACKNOWLEDGEMENTS

We thank Ms. Totsuka, Ms. Yokota, Ms. Ikeda, Ms. Higuchi and for laboratory assistance. We are grateful to Dr. Koutaro Maeno and Dr. Harano Ken-Ichi for kind advice and encouragement at the Locust Research Laboratory, National Institute of Agrobiological Sciences at Ohwashi, Tsukuba. The grass used was raised by Field Management Section of NIASO. This study was supported by the "Japanese Association of University Woman" Research Fellowships.

Abbreviations: W, Whitish body colour of hatchlings; G, grey body colour of hatchlings; B, black body colour of hatchlings; B1, B2, B3 and B4, beige body colour of the last instar nymphs according to the darkness; G, green body colour of the last instar nymphs; B1, beige body colour of adults without black patterns; B2, beige body colour of adults with black patterns; BL, Black body colour of adults; G, grey body colour of adults; Y, yellow body colour of adults; E, length of forewing; F, length

of hind femur; **C**, maximum head width; **H**, maximal height of pronotum; **P**, length of pronotum; **G.F**, gregarious females; **S.F**, solitary females; **G.M**, gregarious females; **S.M**, solitary male

REFERENCES

- Albrecht FO, Verdier M, Blackith RE (1959). Maternal control of ovariole number in the progeny of the migratory locust. *Nature*, 184: 103-104.
- Albrecht FO (1967). Polymorphisme phasaire et biologie des Acridiens migrants. Ed. Masson et Cie, Paris, p. 194.
- Applebaum SW, Heifetz Y (1999). Density-dependent physiological phase in insects, *Ann. Rev. Entomol.*, 44: 317-341.
- Ben Hamouda A, Ammar M, Ben Hamouda MH, Bouain A (2009). The role of egg pod foam and rearing conditions on the phase state of the Asian migratory locust *Locusta migratoria migratoria* (Orthoptera, Acrididae). *J. Insect. Physiol.*, 55: 617-623.
- Bernardo J (1996). Maternal effects in animal ecology. *Am. Zool.*, 36: 83-105.
- Bouaïchi A, Roessingh P, Simpson SJ (1995). An analysis of the behavioural effects of crowding and re-isolation on solitary-reared adult desert locusts (*Schistocerca gregaria*) and their offspring. *Physiol. Entomol.*, 20: 199-208.
- Chapman RF (1998). *The Insects: Structure and Function*. 4th edition. Cambridge University Press, New York, p. 788.
- Chopard L (1938). La biologie des Orthoptères. Encyclopédie entomologique. Ed. Paul Lechevalier, Paris, p. 478.
- Cockereil TDA (1933). The Phases of African Locusts. *Am. Nat.*, 67: 93-96.
- Dale JF, Tobe SS (1990). The endocrine basis of locust phase polymorphism. In: Chapman, R.F., Joern, A. Ed. *Biology of Grasshoppers*. John Wiley and Sons, New York, pp. 393-414.
- Duck LG (1944). The bionomics of *Schistocerca obscura* (Fabr.). *J. Kans. Entomol. Soc.*, 17: 105-119.
- Faure JC (1932). The phases of locusts in South Africa. *Bull. Entomol. Res.*, 23: 293-405.
- Girardie A (1991). Régulation endocrinienne du développement, de la reproduction et du polymorphisme phasaire. La lutte anti-acridienne. Ed. AUFELF-UREF, John Libbey Eurotext, Paris, pp. 119-127.
- Gullan PJ, Cranston PS (2010). *Insects: An Outline of Entomology*, 4th edition. Blackwell Science, 584p.
- Gunn DL, Hunter-Jones P (1952). Laboratory experiments on phase differences in locusts. *Anti-locust Bull.*, 12: 1-29.
- Hägele BF, Simpson SJ (2000). The influence of mechanical, visual and contact chemical stimulation on the behavioural phase state of solitary desert locusts (*Schistocerca gregaria*). *J. Insect. Physiol.*, 46: 1295-1301.
- Hodjat SH (1970). Effects of crowding on colour, size and larval activity of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomol. Exp. Appl.*, 13: 97-106.
- Hunter-Jones P (1958). Laboratory studies on the inheritance of phase characters in locusts. *Anti-locust Bull.*, 29: 1-32.
- Injeyan HS, Tobe SS, Rapport E (1979). The effects of exogenous juvenile hormone treatment on embryogenesis in *Schistocerca gregaria*. *Can. J. Zool.*, 57: 838-845.
- Injeyan HS, Tobe SS (1981). Phase polymorphism in *Schistocerca gregaria*: reproductive parameters. *J. Insect. Physiol.*, 27: 97-102.
- Islam MS, Roessingh P, Simpson SJ, McCaffery AR (1994a). Effects of population density experienced by parents during mating and oviposition on the phase of hatchling desert locusts, *Schistocerca gregaria*. *Proc. R. Soc. Lond.*, 257: 93-98.
- Islam MS, Roessingh P, Simpson SJ, McCaffery AR (1994b). Parental effects on the behaviour and colouration of nymphs of the Desert Locust *Schistocerca gregaria*. *J. Insect. Physiol.*, 40: 173-181.
- Joly P (1968). *Endocrinologie des insectes*. Ed. Masson et Cie, Paris, p. 18.
- Krause J, Ruxton GD (2002). Environmental effects on grouping behavior: Behavioural changes induced by crowding: the desert locust. In: *Living in groups*. Oxford Series in Ecology and Evolution. Ed. Oxford University Press Inc., New York, p. 128.
- Maeno K, Tanaka S (2006). Effects of hatchling body colour and rearing density on body colouration in last stadium nymphs of the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). *Physiol. Entomol.*, 32: 87-94.
- Maeno K, Tanaka S (2008). Maternal effects on progeny size, number and body colour in the desert locust, *Schistocerca gregaria*: density- and reproductive cycle- dependent variation. *J. Insect. Physiol.*, 54: 1072-1080.
- Maeno K, Tanaka S (2009). Artificial miniaturization causes eggs laid by crowd-reared (gregarious) desert locust to produce green (solitary) offspring in the desert locust, *Schistocerca gregaria*. *J. Insect. Physiol.*, 55: 849-854.
- McCaffery AR, Simpson SJ, Islam MS, Roessingh P (1998). A gregarizing factor present in the egg pod foam of the desert locust *Schistocerca gregaria*. *J. Exp. Biol.*, 201: 347-363.
- Norris MJ (1950). Reproduction in the African migratory locust (*Locusta migratoria migratorioides* R. & F.) in relation to density and phase. *Anti-Locust Bull.*, 6: 1-48.
- Norris MJ (1952). Reproduction in the desert locust (*Schistocerca gregaria* Forskål) in relation to density and phase. *Anti-Locust Bull.*, 13: 1-49.
- Papillon M (1960). Etude préliminaire de la répercussion du groupement des parents sur les larves nouveau nées de *Schistocerca gregaria* Forsk. *Bull. Biol. Fr. Belg.*, 94: 203-263.
- Papillon M (1970). Influence du groupement des adultes sur leur fécondité et sur le polymorphisme de leur descendance chez le criquet pèlerin, *Schistocerca gregaria* Forsk. *Colloques Internationaux du Centre National de la Recherche Scientifique L'influence des Stimuli Externes sur la Gamétogenèse des Insectes*, Paris, CNRS, pp. 71-86.
- Pener MP (1991). Locust phase polymorphism and its endocrine relations. *Adv. Insect. Physiol.*, 23: 1-79.
- Pener MP, Yerushalmi Y (1998). The physiology of locust phase polymorphism: an update. *J. Insect. Physiol.*, 44: 365-377.
- Pener MP, Simpson SJ (2009). Locust phase polyphenism: an update. *Adv. Insect. Physiol.*, 36: 1-286.
- Rowell CHF (1967). Corpus allatum implantation and green/brown polymorphism in three African grasshoppers. *J. Insect. Physiol.*, 13: 1401-1412.
- Rowell CHF (1971). The variable colouration of the acridoid grasshoppers. *Adv. Insect. Physiol.*, 8: 145-198.
- Simpson SJ, McCaffery AR, Hägele BF (1999). A behavioural analysis of phase change in the desert locust. *Biol. Rev. Camb. Philos. Soc.*, 74: 461-480.
- Stower WJ (1959). The colour patterns of "hoppers" of the desert locust (*Schistocerca gregaria* Forskål). *Anti-Locust Bull.*, 32: 1-75.
- Tanaka S (2006). Corazonin and locust phase polyphenism. *Appl. Entomol. Zool.*, 41: 179-193.
- Tanaka S, Maeno K (2008). Maternal effects on progeny body size and colour in the desert locust, *Schistocerca gregaria*: examination of a current view. *J. Insect Physiol.*, 54: 612-618.
- Tanaka S, Maeno K (2010). A review of maternal and embryonic control of phase dependent progeny characteristics in the desert locust. *J. Insect Physiol.*, 56: 911-918.
- Uvarov BP (1921). A revision of the genus *Locusta*, L. (= *Pachytylus*, (Fieb.), with a new theory as to periodicity and migrations of locusts. *Bull. Entomol. Res.* 12: 135-163.
- Uvarov B (1966). *Grasshoppers and Locusts*. Cambridge University Press, Cambridge, Vol. 1.
- Uvarov B (1977). *Grasshoppers and Locusts*. Centre for Overseas Pest Research, Vol. 2.