Desiccated desert locust embryos reserve yolk as a "lunch box" for posthatching survival

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Abstract

Oviparous animals often prioritize the allocation of reproductive resources to egg size over clutch size, but the impact of this maternal investment on the allocation of embryonic yolks and its ecological functions is poorly understood. We investigated how desert locust embryos allocate yolk for survival energy after hatching, rather than embryonic somatic growth depending on egg size in response to desiccation stress. Crowd-reared females (gregarious phase) produced significantly larger progeny with higher tolerance to starvation than females reared in isolation (solitarious phase). Abnormally small hatchlings with residual yolk in their gut emerged from small and large eggs when exposed to desiccation. In particular, these small hatchlings of desiccated eggs survived significantly longer under starvation than those of wet ones, with larger eggs providing even greater survival benefits. Physiological analysis showed that hatchlings from desiccated eggs showed a trade-off by reserving more lipids without somatic growth than those from normal eggs. Desiccation could be a reliable signal for embryos to predict future poor vegetation, and reserved energy could increase the chance of accessing food after hatching. Our results underscore adaptive plasticity in maternal and embryonic resource allocation in desert locusts in response to unpredictably variable semi-arid habitats.

Keywords: adaptive strategy, desiccation, embryos, maternal effects, resource allocation

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Significance Statement

Why do some animals increase their egg size under certain conditions? We investigated why gregarious desert locusts lay larger eggs than solitarious ones in the Sahara Desert, where dry, desiccating conditions are widespread. We show that larger progeny survive longer than smaller ones, which is expected. However, hatchlings from desiccated large eggs are abnormally small but have more yolk as energy and survive longer under starved conditions than hatchlings from normal eggs. This reserved yolk functions as a "lunch box" for a trip to reach food resources after hatching. Therefore, producing large eggs is advantageous under harsh conditions. These findings highlight the adaptive significance of plasticity in egg size, maternal and embryonic resource allocation, and adaptation to desert conditions in animals.

Introduction

Resource allocation is necessary to adapt to variable environments when resources are limited (1). Most invertebrates and many vertebrates are oviparous, and mothers package essential nutrients (i.e. yolk) into the egg (2). The yolk serves as a source of nutrients for embryogenesis, as well as an energy reserve for the period between hatching and accessing external food sources (called the "lunch box" strategy) (3–7). In response to unfavorable conditions, many oviparous animals allocate more reproductive resources towards larger egg size rather than producing more eggs (8, 9). Generally, larger offspring from larger

eggs exhibit better survival and developmental performance than smaller ones under adverse conditions, as known as "bigger is better" hypothesis (10). Increasing the amount of yolk reserved in eggs can enhance posthatching survival chances (11). The amount of yolk in the egg is limited, especially for animals that exhibit no parental care and no exogenous nutrition; therefore, the allocation of yolk to either somatic growth or to an energy reserve to be used posthatching could be a critical trade-off for embryos (11). Although embryonic yolk utilization patterns have been examined in many taxa, such as fishes, reptiles, and insects (6, 12), little is known about how maternal and embryonic

resource allocation are jointly used to adapt to variable environments (13).

In terrestrial oviparous animals, eggs may be often subjected to the spatio-temporal desiccation risk if there is no parental care (14). Several adaptive strategies to overcome stress from desiccation in eggs are known, such as diapause and quiescence (15, 16). All adaptive strategies are linked to dry conditions in species-specific habitats and life history (13, 14). Desiccation stress during embryogenesis could reflect future adverse food conditions after hatching. In terrestrial oviparous reptiles, such as turtles (17, 18) and lizards (19), eggs laid in dry conditions produce smaller hatchlings with more reserved yolk than eggs laid in wet conditions. Although it is reasonable to expect that this allocation of embryonic yolks based on moisture conditions serves as a lunch box strategy to cope with future potential poor food conditions caused by drying environments (20), few studies have tested this (21). Here, we investigated how embryos of the desert locust, *Schistocerca gregaria*, plastically reserve yolk in response to desiccation stress.

Desert locusts inhabit semi-arid areas, and their high mobility allows them to reach seasonal breeding sites (22, 23). As immature adults do not start sexual maturation in dry environments, adult diapause has been thought to be the main strategy to overcome variable dry periods (24, 25). Female locusts lay eggs under 2–15 cm of moist soil, and eggs must absorb water from the surrounding environment during the early embryonic stage (26–28). Because quiescence, rather than diapause, has been reported in desert locust eggs, and oviposition has been observed at various sites (29), developing eggs may experience desiccation, and hatchlings will encounter poor food resources.

The desert locust is a notorious pest in >60 countries in Africa and Asia and causes serious agricultural damage (30, 31), so understanding phase polyphenism from an ecological point of view is essential to predict locust outbreaks. Desert locusts show extreme phenotypic plasticity known as density-dependent phase polyphenism; depending on the density of the local population, they can transit between a sedentary solitarious phase and a swarming gregarious phase (32–34). Desert locusts plastically allocate reproductive resources to either egg size or clutch size, depending on local density at the adult stage: gregarious locusts occurring at high density produce fewer but larger eggs than solitarious locusts occurring at low density (35, 36). However, its adaptive strategies against desiccation by producing large eggs associated with phase polyphenism remain unclear.

Soil moisture indirectly triggers the phase transition in desert locusts through vegetation cover and distribution; drying causes patchily distributed vegetation in restricted areas, which causes local crowding, leading to gregarization (37–41). Solitarious females, which usually lay small eggs, can plastically increase egg size in response to crowding (42, 43). Consequently, large eggs laid by transient gregarious locusts in moist soil could experience desiccation at the beginning of the dry season, limiting hatchling access to vegetation (44, 45). It is reasonable to assume that producing large eggs is an adaptive life-history strategy in dry environments in desert locusts.

In a previous study where the egg size could be modified by desiccation, abnormal small green hatchlings appeared from large desiccated eggs instead of large black hatchlings from nondesiccated eggs (46). The hatchlings of this abnormal phenotype contained yolk-like substances in their gut, but the function of this substance was unclear. We hypothesized that desert locust embryos physiologically allocated yolk to posthatchling reserves instead of somatic growth in response to desiccation stress, and

the reserved yolk could function as an energy reserve (that is, lunch box) to be used after hatching. We also predicted that hatchlings from desiccated larger eggs would survive longer than those from desiccated small ones because large eggs have more yolk than small ones. The present study investigated whether maternal gregarization, producing large eggs with greater allocation of embryonic resources and allocation of embryonic yolks, could be a unique adaptive strategy for coping with drying environments in desert locusts.

Results

Maternal reproductive resource allocation and egg characteristics

Crowd-reared (gregarious phase) females produced fewer larger eggs than females reared in isolation (solitarious phase) (Fig. S1; t test, $t = -12.69$, $f = 161$, $P < 0.001$ for egg length; $t = 4.78$, $f = 22.8$, $P < 0.001$ for clutch size). Gregarious eggs produced by crowd-reared females were, on average, 35% heavier than solitarious eggs produced by females reared in isolation (Fig. S2A; t test, $t = -21.98$, $f = 483.2$, $P < 0.001$). Other properties, including dry weight, water content, and lipid content, were also higher in gregarious eggs than in solitarious eggs (Fig. S2B–D). The relative water content was slightly higher in solitarious eggs than in gregarious eggs (Fig. S2E; Wilcoxon rank test, $z = 3.186$, $P < 0.01$), whereas relative lipid content was higher in gregarious than solitarious eggs (Fig. S2F; Wilcoxon rank test, $z = -4.27$, $P < 0.001$).

In the control, eggs kept wet throughout development had hatchability as high as 85% for both solitarious and gregarious eggs, while it was significantly reduced in eggs under desiccation stress on day 5 (Fig. S3). No significant differences in hatchability were observed between solitarious and gregarious eggs (nonparametric multiple Steel–Dwass test, $P > 0.05$).

Starvation tolerance experiment: lunch box hypothesis

Desiccated eggs were shrunken and abnormal, solitarious-like, small phenotypes hatched (Figs. 1 and 2). In solitarious and gregarious hatchlings of normal wet eggs, the gut was filled with small amounts of liquid, and in abnormal hatchlings of desiccated eggs, yolk-like substances were found in the gut (Fig. 2). Typical solitarious green and gregarious black hatchlings appeared from normal wet solitarious and gregarious eggs, respectively
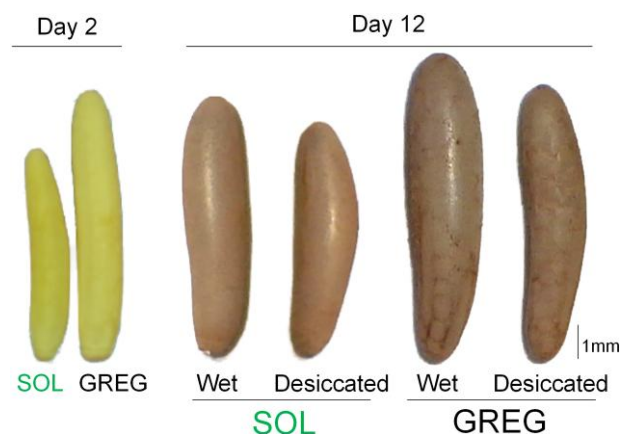
Fig. 1. Solitarious (SOL) small and gregarious (GREG) large eggs exposed to either wet or desiccated conditions.
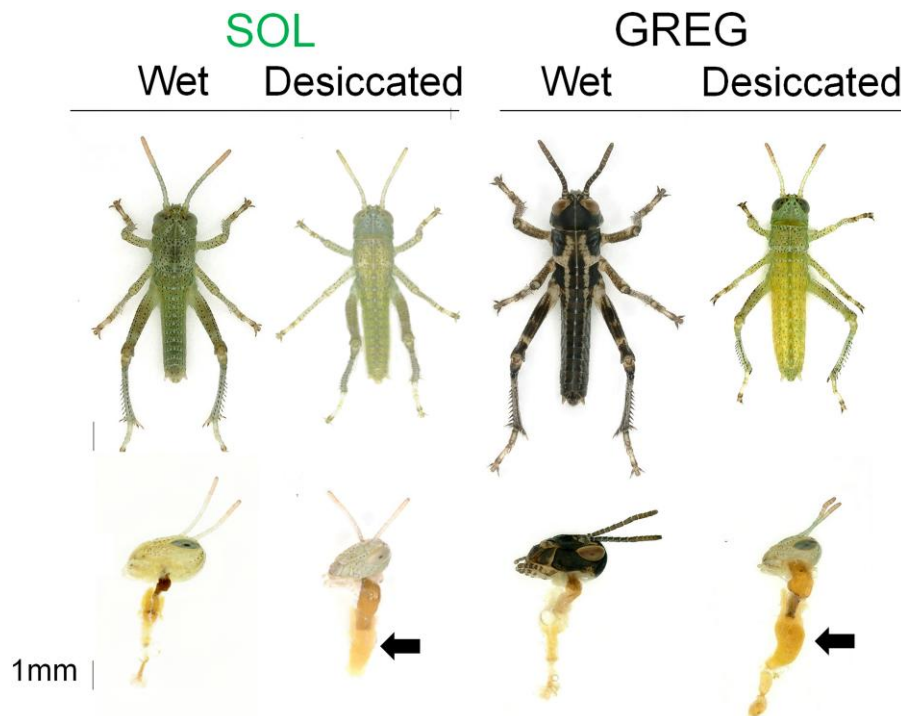
Fig. 2. Hatchlings from either wet or desiccated solitarious (SOL) and gregarious (GREG) eggs. Hatchlings from desiccated eggs have yolk in the gut (arrow).

(Fig. S4). Solitarious eggs from both wet and desiccation treatments produced a high proportion of green hatchlings (Fig. S4). Most hatchlings from normal, wet, gregarious eggs were black, but the proportion of green hatchlings increased significantly when eggs were desiccated ($\chi^2 = 1{,}129.9$, $P < 0.001$). A similar trend was also observed for gregarious eggs from a wild strain originating from Mauritania ($\chi^2 = 173.7$, $P < 0.001$). Freshly hatched nymphs from solitarious eggs were significantly lighter than those that hatched from gregarious eggs (Fig. 3A; Tukey–Kramer HSD test, $P < 0.05$), but hatchlings from desiccated eggs were lighter than the normal phenotype seen in both groups (Tukey–Kramer HSD test, $P < 0.05$). Normal gregarious hatchlings survived significantly longer than solitarious hatchlings under starvation (Fig. 4; Kaplan–Meier test; $P < 0.001$). Hatchlings from desiccated solitarious eggs lived, on average, 65% longer than hatchlings from normal solitarious eggs (76.8 h ± 2.3 and 46.4 h ± 2.7, respectively) under starvation conditions (Kaplan–Meier test, $P < 0.001$). A similar trend was also found for gregarious hatchlings (normal phenotype, 66.5 h ± 2.3; hatchlings from desiccated eggs, 106.9 h ± 2.1), and both groups survived longer than solitarious hatchlings (Table S1). Hatchlings from desiccated gregarious eggs survived 230% longer than those from wet solitarious eggs (Kaplan–Meier test, $P < 0.001$).

The developing embryo encapsulated the yolk during dorsal closure, and the yolk was then within the lumen of the developing gut (Fig. S5). To examine the physiological function of the reserved yolk-like substances in the abnormal phenotype, we produced another abnormal small phenotype lacking most yolk by squeezing yolk from gregarious large eggs (Fig. 5A and B). The hatchlings without reserved yolk died faster than those with yolk (Fig. 5C and Table S2), indicating that reserved yolk increases starvation tolerance.

To determine the substantial starvation tolerance, i.e. the lethal time limit to access food to reach the second stadium, food was provided after various lengths of starvation periods for normal hatchlings from wet solitarious and gregarious eggs and abnormal small hatchlings from desiccated gregarious eggs (Fig. 6). For all groups, the percentages of hatchlings that reached the second stadium gradually decreased as the length of the starvation period increased. Normal gregarious hatchlings showed a slightly longer time limit than solitarious ones, while the abnormal phenotype showed a strikingly longer time limit than normal ones (post hoc Fisher's exact test after Bonferroni correction, $P < 0.000368$). Even after 120 h of starvation treatment, 15% of the abnormal hatchlings reached the second stadium. Longer starvation treatment prolonged the duration of the first stadium (Fig. S6A), but did not significantly influence body size in the second stadium in solitarious and gregarious hatchlings (Fig. S6B). A similar trend was also observed for the abnormal phenotype, but negative effects of delayed development were not observed for groups starved for 24 h. Normal hatchlings did not produce urine during starvation, but abnormal phenotypes produced white-creamy urine after 24 h of starvation, indicating that yolk was consumed.

The abnormal hatchlings of the desiccated large gregarious eggs displayed highly variable weights, which covered the whole range of normal solitarious and gregarious hatchlings (Fig. S7). The smaller abnormal hatchlings tended to survive longer than larger ones ($r^2 = 0.17$, $P < 0.001$), indicating that the preservation of more yolk without somatic growth increases survival time.

Embryonic resource allocation

To examine how desiccated embryos allocate yolk to somatic growth or energy reserve, we measured the water content, dry body weight, and lipid content of hatchlings as a consequence of embryonic development, in addition to fresh body weight (Fig. 3). As expected, the water content was significantly lower in the hatchlings of desiccated eggs than in the hatchlings of wet eggs in both small and large solitarious eggs (Fig. 3B; Tukey–Kramer HSD test, $P < 0.05$). However, no significant differences were observed in
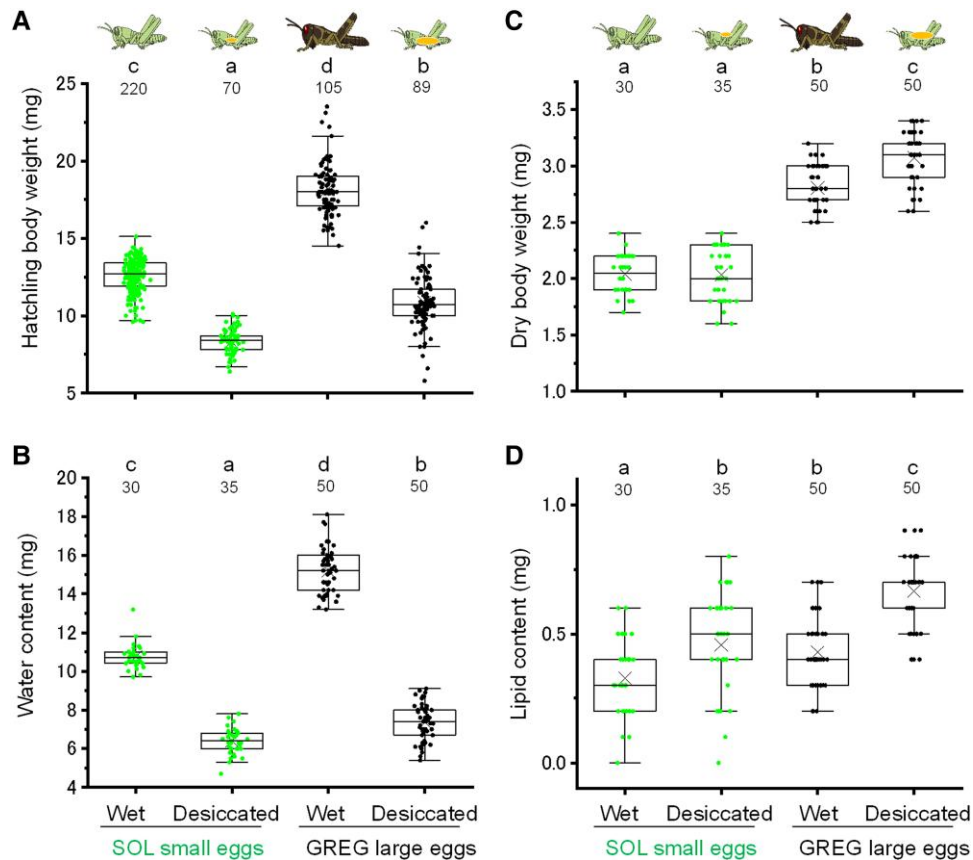
Fig. 3. Physiological traits of fresh body weight (A), water content (B), dry body weight (C), and lipid content (D) from hatchlings from wet or desiccated solitarious (SOL) small and gregarious (GREG) large eggs of desert locusts. Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles, and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey–Kramer HSD test). Numbers in figures indicate sample sizes.
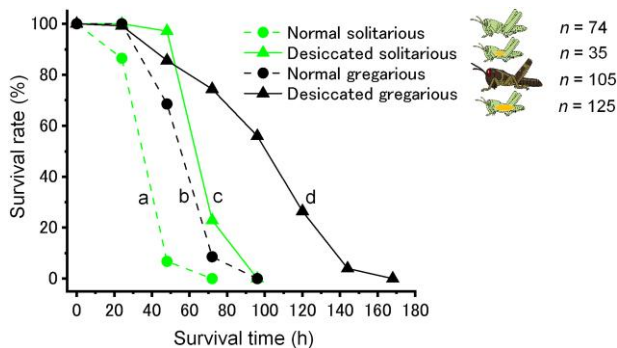
Fig. 4. Survival curve of hatchlings from wet (bullet, dotted line) or desiccated eggs (filled triangle, solid line) derived from solitarious small or gregarious large eggs under starvation. Numbers in figures indicate sample sizes.

dry body weights between hatchlings from solitarious wet and desiccated eggs (Fig. 3C; Tukey–Kramer HSD test, $P > 0.05$). Furthermore, hatchlings from desiccated gregarious eggs were slightly heavier than those from wet gregarious eggs (Fig. 3C; Tukey–Kramer HSD test, $P < 0.05$). The lipid content was significantly higher in the abnormal phenotype than in the normal one in both solitarious and gregarious eggs, but it was higher in gregarious eggs than in solitarious eggs (Fig. 3D; Tukey–Kramer HSD test, $P < 0.05$), indicating that embryos from desiccated gregarious eggs reserved more yolk than those from wet and solitarious eggs.

If the reserved yolk of the hatchlings is metabolized as survival energy during starvation, a significant reduction could be found in dry body weight and lipid content after starvation; therefore, dry body weight after starvation would be lighter in hatchlings from desiccated eggs than in those from wet eggs because the former have higher lipid content. To examine these predictions, dry body weight and lipid content were measured before and after starvation for three groups, including normal solitarious and gregarious hatchlings and abnormal hatchlings from desiccated gregarious large eggs (Fig. 7). Dry body weight decreased after starvation for all groups (Fig. 7A; Tukey–Kramer HSD test, $P < 0.05$), indicating that the energy reserved at hatching was metabolized during starvation. Dry body weight before starvation was significantly heavier in abnormal hatchlings than in gregarious controls, while it decreased significantly after starvation (Fig. 7A; Tukey–Kramer HSD test, $P < 0.05$). A significant reduction of lipid content after starvation was observed in all groups (Fig. 7B; Tukey–Kramer HSD test, $P < 0.05$). No significant differences were observed in lipid content after starvation for three groups (Fig. 7B; Tukey–Kramer HSD test, $P > 0.05$), indicating that most of the reserved energy was consumed by the time they died from starvation.
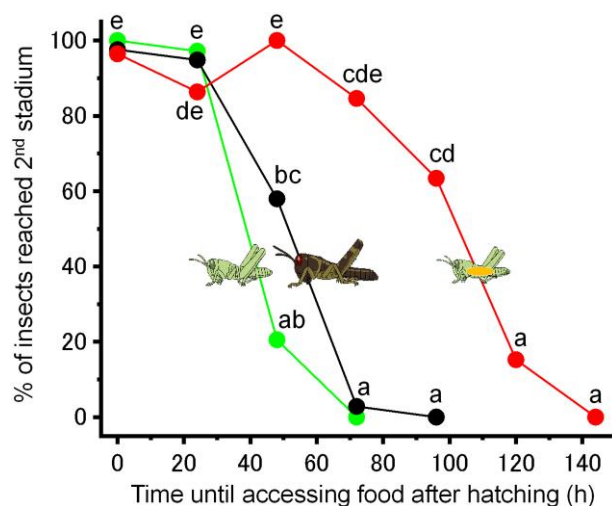
Discussion

The results of this study supported our hypothesis that desiccated embryos allocated more yolk to energy reserves for posthatching than for somatic growth. We found that this lunch box strategy
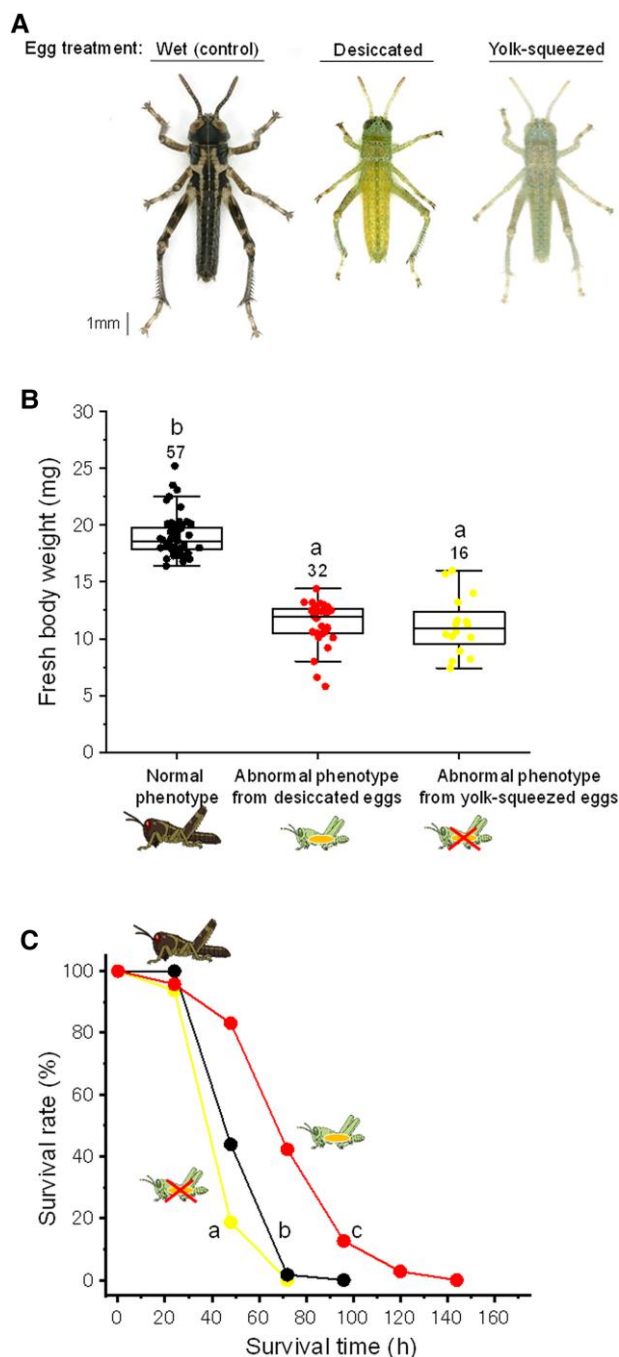
Fig. 5. A) Photographs of hatchlings, B) fresh body weight, and C) survival rate for normal wet small and large eggs and hatchlings from large eggs after yolk removal in desert locusts. Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey–Kramer HSD test). Numbers in figures indicate sample sizes.

Fig. 6. Percentage of insects that reached second stadium after various lengths of starvation from wet solitarious small or gregarious large eggs and desiccated large eggs in desert locusts. Different letters above each circle indicate significant differences at $P < 0.000368$ by post hoc Fisher's exact test with Bonferroni correction. Sample sizes range from 19 to 70 at each circle.

under adverse food conditions. Therefore, our study provides information on how desert locusts plastically adapt to variable moisture conditions through resource allocation. Adaptive traits of life history are shaped by various components, including life stages, habitats, environmental conditions, season, phenotypic plasticity, available resources, mobility, breeding systems, and food preference.

Lunch box strategy and allocation of resources

Our egg desiccation experiments revealed that egg size did not significantly influence hatchability in terms of desiccation tolerance, but high mortality occurred under desiccated conditions. Eggs on dry substrates lost more water during the course of embryogenesis than those on wet substrates. Interestingly, both desiccated solitarious small and gregarious large eggs produced solitarious-like small green hatchlings in appearance. A key finding was that abnormally small hatchlings from desiccated eggs survived considerably longer than normal hatchlings from wet eggs and that starvation tolerance depended on the size of the eggs. In solitarious small eggs, the normal phenotype survived an average of 46.4 h of starvation, while the abnormal phenotype survived 76.8 h; in gregarious large eggs, the normal phenotype survived for an average of 66.5 h of starvation, while the abnormal phenotype survived 106.9 h. In contrast to the general trend that larger hatchlings survive longer than smaller ones (10, 47), these results showed that miniaturized small hatchlings from desiccated eggs survived longer than normal large hatchlings from wet eggs under poor food conditions.

Our dissection revealed that the gut of the hatchlings of the desiccated eggs was filled with residual yolk. It cannot be determined whether a reduction in body size or an increase in the amount of reserved yolk caused by desiccation treatment was the reason for the increased tolerance to starvation. The removal of yolks from eggs is one of the physiological techniques used to determine the function of reserved yolk (48). We also artificially produced small abnormal hatchlings by surgically removing the yolk from large eggs. Small phenotype hatchlings died earlier

was associated with maternal investment. Gregarious mothers allocated more reproductive resources to egg size than solitarious ones by reducing the clutch size, and hatchlings from desiccated gregarious large eggs reserved more yolk and showed greater tolerance to starvation than those of solitarious small eggs. This two-step system of allocation of maternal and embryonic resources could increase the probability of progeny accessing food, even
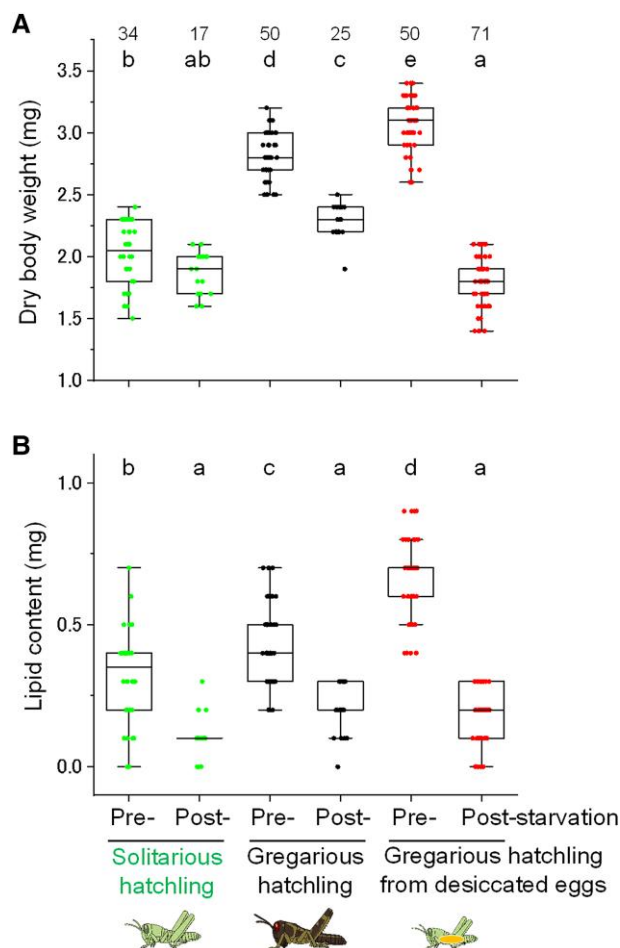
Fig. 7. Physiological changes in dry body weight A) and lipid content B) associated with starvation in hatchlings produced by solitarious and gregarious eggs in desert locusts. Each variable was measured pre- and poststarvation. Numbers in a figure indicate sample sizes. Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles, and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey–Kramer HSD test). Numbers in figures indicate sample sizes.

than normal solitarious ones (ca. 50 h) under starvation conditions; therefore, reserved yolk, rather than miniaturization of body size, is the main reason for increased tolerance to starvation. These results indicated that the reserved yolk served as a lunch box energy supply (5), as observed in yolk-feeding fishes (11).

Our physiological analysis showed that larger eggs had more yolk than smaller ones; the larger amount of reserved yolk in the desiccated large eggs could further increase survival. If death was due to desiccation under starvation, it would be expected that the insects would die before they could consume the lipid. However, in our data, the amount of lipid decreased at the time of death. In our starvation experiments, we supplied a piece of moist tissue paper to prevent death due to drying. Therefore, it is expected that the death was not due to desiccation.

Previous studies have focused on the change in hatchling size that occurs under desiccation conditions for turtles (17, 18). However, the ecological function of the reserved yolk by desiccation remains unclear. By using gregarious large locust eggs, we determined the lethal time limit to be accessing food after hatching to reach the second stadium. We found that normal gregarious

hatchlings needed to reach food before ~3 days, while abnormal hatchlings from desiccated eggs could wait until ~5 days. Although the duration of the first stadium period increased as the starvation period became longer in all phenotypes, no significant delay was found for small hatchlings from desiccated large eggs after 24 h of starvation. This result indicated that the reserved yolk was used in the same way as the food consumed in a day. Our rearing experiments also showed that body weight in the second stadium was not significantly influenced by the duration of starvation. However, body weight in the second stadium was heavier in wet egg hatchlings than in desiccated ones. In desert locusts, nymphs can molt when they reach a certain critical body weight, and smaller hatchlings tend to develop into smaller second-stadium nymphs (49, 50); thus, initial small body size caused by desiccation may cause small body size in the second stadium. No significant increase in body weight was observed in nonstarved hatchlings from desiccated large eggs. These results suggested that the reserved yolk was used not only for survival energy but also as an energy resource to be consumed on the first day and not as extra nutrition to become larger nymphs.

The present study also found that energy reserves in desert locust embryos are regulated through a trade-off mechanism, reducing yolk allocation to somatic growth under desiccation stress. Physiological analysis showed that hatchlings from desiccated large eggs were lighter at hatching compared to those from wet eggs, despite having heavier dry body weights, as observed in reptiles (17, 18). After starvation, hatchlings from desiccated eggs had lighter dry body weights, indicating that they had used more consumable energy substances. Yolk lipid content was higher in desiccated eggs hatchlings, which decreased significantly after starvation, suggesting that it was used up during starvation. This indicates that desert locust embryos plastically adjust resource allocation in response to desiccation stress, prioritizing energy reserves over somatic growth. This allocation of embryonic yolks is continuous because desiccated gregarious large eggs produced hatchlings of various body sizes, and smaller hatchlings tended to survive longer than larger ones.

Animals living in highly variable environments must overcome unfavorable conditions (14). Migration allows animals to escape adverse environments and reach new habitats (51), while diapause allows animals to overcome unfavorable dry periods (16). The yolk reserved in juveniles just after hatching serves as a source of survival energy for the period between hatching and accessing external food sources, as called the lunch box strategy (3–7). The lunch box strategy has also been proposed as an effective way to adapt to adverse environments at the earliest life stage (3, 5). Maternal provisioning is believed to have evolved in response to variability and unpredictability in the quality of the offspring environment (52), and plasticity has been proposed as an adaptive strategy to survive variation in environmental conditions (52–55). The lunch box strategy of desert locusts through an integration of maternal and embryonic plastic resource allocation is a newly discovered adaptive strategy to semi-arid habitats that are unpredictably variable.

Adaptive importance of phase polyphenism to semi-arid areas

Although the most economical strategy resulting in the highest fitness return for a mother could be to plastically produce clutches of appropriately sized eggs in response to variable environments (8), physiological and morphological characteristics generally constrain this ability. On the contrary, desert locusts

can respond to variable environments through their plasticity of phase polyphenism. Although our information on natural conditions is still limited, in the following we try to trace the different environmental conditions that desert locusts meet in different phases.

Solitarious locusts can wait as adults to reproduce when environmental conditions are favorable (24). Solitarious females lay many small eggs, but eggs are guaranteed access to moist soil during embryogenesis (24–26). We confirmed that small solitarious hatchlings are short-lived when deprived of food, but they normally hatch in damp and marshy areas, where food is likely to be abundant during the rainy season (56).

After a short rainy season, reducing vegetation cover could concentrate widely scattered solitarious adults in restricted areas (37–41). Laboratory experiments showed that solitarious locusts, which lay many small eggs, plastically increased egg size and reduced clutch size when forced into gregarious conditions, even at the late adult stage (42, 43). Such physiologically gregarized solitarious locusts (transient phase) could, through local crowding, begin to lay large eggs equivalent to the large gregarious eggs at the beginning of the dry season in the field (45). Physical contact between locusts is the main trigger inducing maternal gregarization (57, 58), and this tactile stimulus could be a reliable indicator to reflect drying environments. The group oviposition of transient females occurs after dusk, and this synchronizes the hatching timing when the soil is moist enough. The next generation of hatchlings may encounter environments that are beginning to dry out. Numerous synchronous and large gregarious hatchlings form groups and begin to migrate, regardless of local vegetation conditions, and complete development before all vegetation dries up (59, 60). We confirmed that gregarious hatchlings showed better tolerance to starvation than solitarious ones (47, 61). Gregarious large hatchlings that survive for a relatively long period of time may sometimes have to search for food. The nymphal period is shorter in gregarious hatchlings than in solitarious ones, so the former can quickly reach a highly mobile adult stage, making them more adaptive under vegetation drying conditions (62). Thus, diverting maternal reproductive resource allocation to egg size rather than clutch size leads to an increase in survival after hatching. Maternal allocation plasticity is adaptive in this context.

Later in the dry season, when soil moisture decreases, gregarious large eggs experience desiccation stress. Once they reach this dry stage, unlike the gregarious hatchlings that travel long distances in groups to feed on scantily distributed food grass, group oviposition becomes less likely, and the hatchlings may not gather in groups. We showed that large eggs responded to desiccation stress by reserving yolk and that, under starvation, abnormal small hatchlings that reserved yolk survived longer than normal large hatchlings from wet eggs. We confirmed that Mauritanian wild desert locusts possess this plasticity. In our study, we broke up the egg pods and desiccated them in a Petri dish to efficiently induce abnormally small hatchlings, so that intact egg pods could show better tolerance to desiccation. Desiccation stress during embryogenesis directly reflects future unfavorable environments. Therefore, maternal and embryonic plasticity that increases energy reserves would give longer surviving offspring more time to search for food. Gregarious locusts producing solitarious phenotypes under drying conditions seem like a counterintuitive strategy from a future egg production standpoint. It will be interesting to know how small hatchlings, which have increased starvation resistance, move to reach feeding sites. We expect that this lunch box strategy is a backup function to increase the probability of survival over a short period when the soil is in near-completely dry conditions. The embryonic plasticity is adaptive in this context.

Therefore, the phase polyphenism of desert locusts at different developmental stages confers strong adaptive plasticity to an unpredictably variable environment. The eggs of the gregarious phase are more adapted to a variable environment with potentially very harsh conditions. From an evolutionary point of view, adaptive phase polyphenism could have been selected only as a response to conditions varying from sometimes very favorable (moist) to very harsh (dry) (63). As predicted by previous studies (64), understanding the ecological traits of locusts to adapt to varying habitats is essential to predict population dynamics and outbreaks (65). The present study demonstrates an overlooked ecological significance of gregarization and special desert adaptation in the embryonic plasticity of desert locusts.

Materials and methods
Study animals
The desert locust individuals studied in the present article were a Mauritanian strain kept in the CBGP. Nymphs and adults were kept in groups of ~100 individuals in large cages ($40 \times 40 \times 42$ cm) or isolated in small cages ($12 \times 12 \times 10$ cm) at $32 \pm 1\,°C$, with a 12:12 h light:dark photoperiod under 20–40% relative humidity, in a well-ventilated room. They were fed fresh wheatgrass leaves and wheat bran.

Egg pod collection and measurement of egg size and egg number
Desert locusts produce typical solitarious and gregarious hatchlings depending on the rearing density during the adult stage, regardless of the rearing density before the mother reached the adult stage (i.e. nymphal stage) (42, 66, 67). In this study, eggs and hatchlings derived from isolated and crowd-reared females will be termed solitarious and gregarious, respectively. Female locusts reared under crowded conditions, as nymphs were placed in crowded conditions (in a large cage) or isolated in small boxes after adult emergence. Plastic cups (diameter, 5 cm; height, 10 cm) filled with clean, moist sand were placed in the cages to collect the egg pods. The egg pods collected were incubated at $32 \pm 1\,°C$. On day 2, each egg pod was washed with tap water to remove sand and frothy secretion, and individual eggs were gently placed on moist tissue paper in Petri dishes (9 cm in diameter; 1.5 cm in height) to avoid desiccation before measurements. To examine the relationship between egg size and clutch size, a total of 10 eggs were randomly chosen from each egg pod, and the egg length was measured to the nearest 0.1 mm using an ocular micrometer installed in a stereo microscope. The mean lengths of 10 eggs were used as the egg size. The number of eggs per clutch was recorded at the same time. Egg pods were collected from 51 isolated females and ~80 females raised in a crowd. In isolated conditions, the order of egg pods laid by individual females ranged from 1 to 4 were used; however, it was difficult to follow the oviposition history of females in crowded conditions. During daylight hours, oviposition was checked ~3 h and collected. We collected egg pods from crowded conditions when the females were between 20 and 40 days old.

Physiological characterization of eggs
To determine the physiological characteristics of solitarious and gregarious eggs, fresh body weight, dry body weight, water content, and lipid content were examined using solitarious and

gregarious egg pods. Samples of 10 eggs per clutch from both groups were randomly weighed on day 2 after oviposition, and then immediately placed in sealed vials in a freezer (20 °C). The eggs were then oven-dried at 60 °C for 2 days, weighed again and placed in 1.2 mL of chloroform/methanol (2:1) solution for 4 days at 25 °C, during which the solution was changed once, according to the method of Maeno et al. (47). Total water and lipid content was calculated by the difference between fresh body weight and dry weight, and between the dry weight and the lean dry weight, respectively. The percentage of water and lipid content to body size was calculated by water content/fresh body weight and lipid content/dry body weight, respectively. Eggs broken during a procedure were excluded from the analysis. To avoid biasing data collection due to eggs laid by a particular female, we sampled no more than 15 individuals from a single clutch in each experiment.

Desiccation experiments

Figure 8 shows the experimental design of the present study. Egg pods derived from isolated or crowd-reared females were used for two treatments (for each egg pod), desiccation or wet treatment (control), to determine the tolerance to desiccation of the eggs. Twenty eggs were placed on dry or wet filter paper (9 cm in diameter) with 1 mL of distilled water in a Petri dish on day 5. When the clutch size was <40, the same number of eggs was used for both treatment and control. The eggs in Petri dishes were then held in an airtight plastic container in which relative humidity was kept close to 100% by a moist tissue paper kept in a corner of the container. To maintain adequate levels of moisture in the wet filter paper, water was added as needed. The eggs were covered with moist sand (~1 cm) on day 12 and checked for hatching. Most hatching occurred on days 14–16 after oviposition. All hatchlings were recorded to determine mortality. The hatchlings obtained were used for starvation experiments or physiological analysis, as described below. The body color of the hatchlings was also observed 6 h after hatching. The nymphs were divided into five hatchling color grades (HCGs 1–5), according to visual estimation according to Maeno et al. (42): HCG 1, green body color without dark spots; HCGs 2–4, increasingly darker body color; HCG 5, almost entirely black body color (Fig. S4). Body coloration of all hatchlings was recorded on days 14–18 after oviposition.

To examine whether the eggs of the wild strain also respond to desiccation treatment by reserving the yolk, we collected wild egg pods produced by transient populations in October 2022 located near Akjoujt, northwest Mauritania. The newly laid egg pods were collected from a group oviposition site (20°00N, 14°15′W) in the morning and transported to the Mauritanian National Desert Locust Center (CNLA) to perform desiccation experiments. The same experimental procedure as described above was applied for the wild strain. Five egg pods were randomly mixed and used for two treatments: either desiccation or wet treatment (control). Twenty eggs were placed on dry or wet filter paper (9 cm in diameter) with 1 mL of distilled water in a Petri dish on day 5 or 6, and kept at 31 ± 2 °C. A total of 10 replicates were made. The body color of the hatchlings was also observed 6 h after hatching.

Starvation experiments

Solitarious and gregarious hatchlings and abnormally small hatchlings from large desiccated eggs were housed individually in plastic cylinders (diameter: 3 cm, height: 4 cm) with a piece of moist tissue paper after being weighed on the day of hatching. The survival rate was recorded at 10:00 h ± 1 h every day to determine the tolerance to starvation. Individuals that did not move at all were considered dead. To examine the substantial starvation tolerance to reach the second stadium, these insects received cut fresh wheatgrass leaves twice a day at 0, 24, 48, 72, 96, 120, and 144 h after starvation treatment. The upper limit was set at 144 h because most of the animals died after 144 h, and it would be difficult to ensure a sufficient sample size. Mortality and molting at the second stadium were recorded every day. Normal solitarious and gregarious hatchlings did not survive as long as abnormal ones, so we used a starvation treatment of 0, 24, 48, and 72 h for solitarious hatchlings and of 0, 24, 48, 72, and 96 h for gregarious hatchlings. The experiments were carried out in the same rearing room as described above. Hatchling body size and coloration are continuous in desert locusts, and desiccated large eggs sometimes produced small black hatchlings (46). In these main experiments, we used only typical small, abnormal green hatchlings from desiccated eggs (only insects that had yolk in the gut). We also carried out the same starvation experiments using hatchlings of various sizes from desiccated gregarious eggs.

Measurements of the water and lipid content of hatchlings

Lipids are the main source of energy during the early posthatch period in desert locust (47). Two groups were used to measure the total water and lipid content: hatchlings before starvation and hatchlings after starvation. Individuals from both groups were weighed on the day of hatching. The hatchlings of the first group were then immediately placed in sealed vials in a freezer (–20 °C). Hatchlings from the second group that died after 48 h were used in the analyses to only consider insects that actually died of starvation. These were placed in the same freezer on the day of death. The insects were then weighed, dried in an oven at 60 °C for 2 days, weighed again, and then placed in a solution of chloroform:methanol (2:1) of 1.2 mL for 4 days, during which the solution was changed once, according to the method described by Maeno et al. (47). The total water content and lipid content were calculated by the difference between the fresh body weight and the dry weight, and between the dry weight and the dry weight after lipid extraction, respectively.

Miniaturization of eggs and starvation experiments

To produce abnormal small hatchlings without reserved yolk from large gregarious eggs, the yolk was squeezed out of the eggs, according to the method described by Maeno and Tanaka (46). Five days after oviposition, eggs immersed in phosphate-buffered saline solution (T900, Takara Co.) were pricked with a needle near the posterior end. The embryos in these eggs were in the anatrepsis stage, located near the anterior end of the egg (27). Different amounts of yolk could be squeezed out without damaging the embryo by gently pressing the eggs with a pair of tweezers. After the removal of some yolk, the eggs were rinsed with saline solution, and the injured portion was air-dried for half an hour at 22 °C. The eggs were then put back in contact with wet filter paper in Petri dishes for further incubation under the same conditions described above. The hatchling nymphs were removed from the dishes every day and weighed. Sixteen small abnormal hatchlings were used for the starvation experiment. Normal phenotype hatchlings from large wet eggs and abnormal small ones from large desiccated eggs were used as controls. Survival rate was recorded at 10:00 h ± 1 h every day to
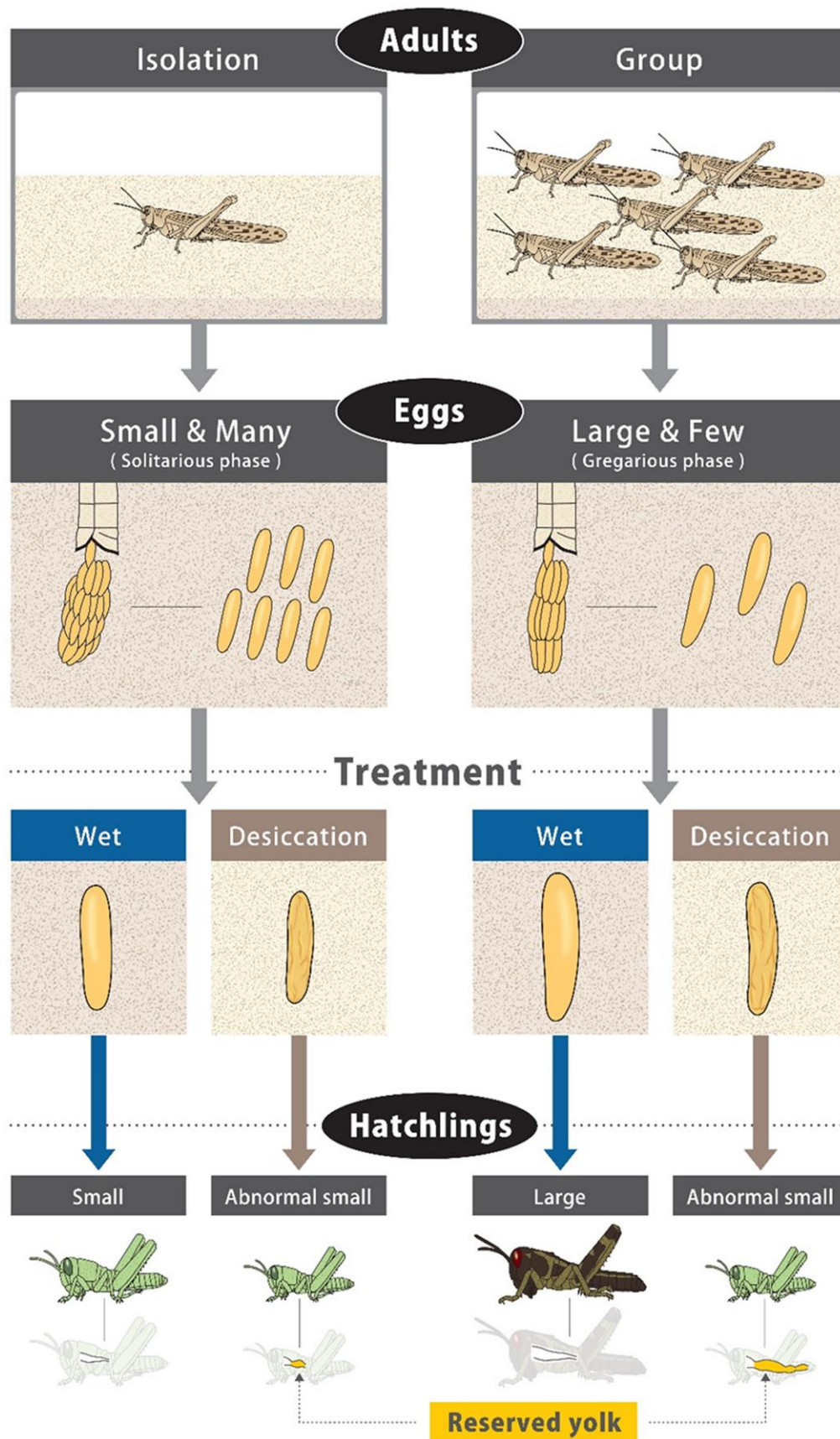
Fig. 8. Experimental design. Egg pods derived from isolated- or crowd-reared females are used for two treatments: either wet (control) or desiccation treatment. Crowded-reared locusts lay larger but fewer eggs than isolated-reared ones. Eggs and hatchlings derived from isolated and crowd-reared females will be termed solitarious and gregarious, respectively. Typical phenotypes of hatchlings are illustrated with their reserved yolk. Please see Materials and methods for details.

determine the tolerance to starvation as described above. Miniaturizing small eggs was a technical difficulty. If we remove the yolk from the small eggs, they do not develop normally.

Statistical analysis

The characteristics of the egg and hatchling, including length, fresh and dry weight, and number of eggs per pod, were compared between egg pods derived from females reared in isolation and from females raised in a crowd using a t test or Tukey–Kramer HSD test following an ANOVA. Hatchability and differences in percentages of water and lipid content relative to body weight were analyzed using a nonparametric Steel–Dwass test. The survival rates of the hatchlings were compared using a Kaplan–Meier test. The percentage to become second-stadium nymphs after starvation was subjected to a post hoc Fisher's exact test after Bonferroni correction. R software package, version 4.2.3 (68) and JMP (SAS Institute, Cary, NC, USA) were used for the analyses.

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Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Author Contributions

K.O.M. designed and performed the research and analyzed the data. K.O.M. and C.P. wrote the paper. C.P. and N.L. provided the resources. C.P., N.L., S.O.E., M.A.O.B.E., A.S.B., and M.E.H.J. reviewed the final manuscript. S.O.E., M.A.O.B.E., A.S.B., and M.E.H.J. organized the field surveys.

Data Availability

Raw data are available on figshare: https://figshare.com/s/2d65f58fc19199f04f14.

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