This work is on a Creative Commons Attribution 4.0 International (CC BY 4.0) license, https://creativecommons.org/licenses/by/4.0/. Access to this work was provided by the University of Maryland, Baltimore County (UMBC) ScholarWorks@UMBC digital repository on the Maryland Shared Open Access (MD-SOAR) platform.

Please provide feedback

Please support the ScholarWorks@UMBC repository by emailing scholarworks-group@umbc.edu and telling us what having access to this work means to you and why it’s important to you. Thank you.
The Effects of Population Density on the Incidence of Developmental Deformities in Chemosensory Organs of Tobacco Hornworm Larvae (Lepidoptera: Sphingidae)

Frank Hanson,1,3 Elizabeth Stanwyck,2 and Alexander Bohorquez1

1Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD 21250, 2Center for Interdisciplinary Research and Computing, University of Maryland, Baltimore County, Baltimore, MD 21250, and 3Corresponding author, e-mail: hanson@umbc.edu

Received 14 December 2019; Editorial decision 5 June 2020

Abstract

Cultures of Manduca sexta Johanssen in our laboratory were found to have larvae with missing or deformed mouthparts or antennae. Hypothesizing that these developmental deformities were caused by crowded rearing conditions, we reared larvae in four different population densities and recorded the incidence (% of larvae affected) and types of chemoreceptor deformities. Results showed that the incidence of these deformities was directly proportional to larval population density. Deformities of the maxilla and palp were the most frequent, followed by those of the antenna, epipharynx and maxillary styloconica. Life history traits of larval mass, food consumption, and rate of development were inversely related to larval density for both normal and deformed larvae. We discuss possible causes and mechanisms of these deformities and of changes to life history traits.

Key words: larval crowding, chemosensilla, chemosensory deformation, life history traits, larval culture

Larvae of oligophagous insects such as the tobacco hornworm, Manduca sexta Johanssen, rely primarily on their gustatory chemosensory organs for food selection. The main gustatory organs border the chewing mouthparts (mandibles). Dorsal to these mandibles and covering the upper portion of the oral cavity is a bi-lobed flap, the epipharynx (Fig. 1A, arrow); in each lobe is an extremely small sensory organ that is in continuous contact with the oral contents during feeding. The mouthparts just ventral to the mandibles are the bilateral maxilla (Fig. 1A, asterisk), each of which contains two small (~50 µm) styloconica and a palp (Fig. 1A inset, S and P, respectively). The maxillae are highly mobile and can be seen moving into and out of the bubble of plant juice two or three times per second while the animal is feeding. Each of the above sensilla plays a distinct role in acceptance of host plants or rejection of non-hosts (de Boer 2006). Bilateral ablation of all of these sensilla essentially eliminates the animal’s ability to discriminate between host and non-host plants; for example, chemosensory-ablated larvae will eat Canna generalis, a plant so deterrent that normal larvae would prefer to starve rather than eat it (Waldbaur and Fraenkel 1961, Hanson and Dethier 1973, de Boer and Hanson 1987, de Boer 1991). Unilateral ablation also results in a loss of discrimination, but to a lesser degree (de Boer 1991, Flowers and Yamamoto 1992).

The olfactory organs are the antennae (Fig. 1A, diamond) and possibly the maxillary palpi. The antennae have been shown to mediate behavioral attraction to humidity (Rowley and Hanson 2007) and food plants (Glendinning et al. 2009). The lateral maxillary styloconica have near-range (≤600 µm) olfactory capabilities as shown electrophysiologically (Städler and Hanson 1975), but it is not known whether this contributes to the animal’s overall olfactory capability.

Our research on the sensory basis of food selection has traditionally employed laboratory-reared larvae that were screened to exclude those with missing or deformed chemosensory organs described above. The incidence (% of larvae deformed) was noticeably variable, which prompted an investigation to determine the sources of this variability and what might be causing these deformities. After eliminating several potential sources such as genetic variation and hazards to eggs during shipment, we hypothesized that these deformities might be linked to population density in the larval cultures. Here, we present results of experiments to test this hypothesis and determine if any life history traits are also affected by population density. We discuss possible mechanisms and compare results with those from other species.

Methods

Insects

Eggs were obtained from the North Carolina State University Insectary’s M. sexta culture that was initiated in the 1960s and maintained on artificial diet (Yamamoto 1969). These eggs were...
shipped overnight in an insulated container, disinfected with 0.05% bleach solution, rinsed with distilled water, air dried, and placed on plastic mesh (window screen) on the floor of a 12-cm Petri dish. The cluster of eggs was surrounded by strips of tobacco hornworm diet (Yamamoto 1969; commercial source: Frontier Agricultural Sciences, Newark, DE) ~2 cm away to prevent inhibition of hatching. This culture was maintained for eggs and first instar in 16-h light and 8-h dark (L16:D8) and 24°C. Larval instars were determined by comparing head capsules with a ‘library’ of five sizes of head capsules accumulated over the years of rearing this insect. The transition to fifth (final) instar is conspicuous because the head capsule of the fourth-instar premolt appears as a bubble on the much larger fifth-instar head capsule developing behind it (‘bubblehead’).

Population Density Experiments

Newly molted second-instar larvae were transferred to metal wire mesh platforms placed 2 cm above the floor of translucent polypropylene culture boxes (28L × 14W × 10H cm). Small holes in the sides provided passive ventilation. Each day, strips of diet in excess of anticipated consumption were placed on the platforms, and both the uneaten and replenishing food were weighed for the calculation of consumption. These weights were recorded daily for each box, as were larval counts (for the initial number, see below). Culture boxes were cleaned daily and sterilized with dilute chlorine bleach.

The first experiment consisted of 10 sequential trials, each of which was comprised of four simultaneous cultures of different population densities (10, 40, 60, or 100 animals per box) at L16:D8 and room temperature of 22°C, well within the range (20–30°C) for normal weight attainment (Moore et al. 2020). Upon reaching the final premolt condition at the end of the fourth instar, each larva was removed from the culture and transferred to a compartment (5.3 × 5.3 × 5 cm) of a larger transparent plastic box (32 × 21 × 5 cm) maintained at 30°C, 70% humidity, and L16:D8 without food or water until it molted into the fifth instar (usually that night). The larva was then weighed and examined under a dissecting microscope for deformed or missing chemosensory organs. Six classes of chemosensory deformities were recorded (Fig. 1): epipharynx (missing half); maxilla (missing or deformed and without chemoreceptors); maxillary palp (missing or deformed); maxillary styloconica (one or both missing); antenna (missing or deformed); and multiple (more than one of the above). This information was recorded along with the date and its population density cohort; the animal was then euthanized or processed for scanning electron microscopy (SEM).

In the initial trial of this experiment, we only recorded whether each larva was normal or deformed; in the subsequent nine trials, we also recorded the status of each larva’s chemosensilla (normal or type of deformity) and the daily food consumption of each culture box. Thus, analyses involving total incidence of deformities used the data from all 10 trials (1,940 surviving larvae), whereas analyses concerning types of deformities or food consumption used data from only the last 9 trials (1,744 surviving larvae). (Two analyses, consumption and development, were limited to 1,743 and 1,734 larvae, respectively, because of missing data.) The effects of population density on both the incidence and type of chemosensory deformities, larval mass, development time, and food consumption were determined.
A second experiment was done to disentangle the effects of area population density, such as physical interactions between larvae on the feeding platform, versus the effects of volume population density, such as the headspace containing volatile chemicals such as pheromones. This experiment compared the incidence of deformities in cultures having the same populations and air volumes, but different areas of feeding platforms on which the larvae were reared. High-density cultures were reared on normal single feeding platforms (area = 392 cm²), whereas the low-density cultures spread the same number of larvae over three feeding platforms stacked 2 cm apart forming a three-level tier (tier platforms, area = 1,176 cm²). Thus, the latter had one-third of the area density of the former but, because all cultures used the standard culture box, they had the same volume density. A trial consisted of one high-density and three low-density cultures running concurrently at room temperature (~22°C) and light regimen (L16:D8). Three trials were conducted.

Scanning Electron Microscopy
Fixation, ultrasonic cleaning in dilute detergent, and heavy metal deposition were performed on normal and deformed larval heads using standard techniques for SEM. Visualization was done on a JOEL model JSM 5600 scanning electron microscope.

Analysis and Statistics
The incidence (% of all deformed larvae) of the six types of deformities within each population density cohort was compared across the four cohorts (Fig. 2) using the chi-squared test of independence. The incidence of deformities of survivors, and the incidence of mortality, were compared across the four population densities for all 10 trials using linear regressions (Fig. 3A; Table 1). In the tier platform experiment (Fig. 3B; Table 2, A), a t-test was used to compare the incidence of deformities in cultures reared on tier platforms versus single platforms, whereas analysis of variance (ANOVA) was employed to determine whether the incidence of deformities differed among tier platforms (Fig. 3B; Table 2, B). The relationships between larval mass versus population density, and larval mass versus deformities, were analyzed using linear regressions (Fig. 4A; Table 3). The relationship between deformity status of chemoreceptors (normal or type of deformity) and larval mass was tested using ANOVA (Fig. 4B; Table 4, A) and the relationships between specific deformities and larval mass were tested using ANOVA with Tukey’s multiple contrasts (Fig. 4B; Table 4, B). Food consumption across day of culture and population density cohorts was analyzed using linear regression (Fig. 5; Table 5). To determine whether there was a relationship between duration of development versus population density and/or deformities, we compared the percentage of larvae remaining in the culture boxes on days 6–14; the Kaplan–Meier method for analysis of survival curves and the log rank test was used to determine differences (Fig. 6A; Table 6, A). ANOVA was used to test whether duration of development varied with deformity status; ANOVA with Tukey’s multiple contrasts was used to determine if duration of development varied with each specific type of deformity (Fig. 6B; Table 6, B and C).

Results
Chemosensory Organ Deformities: Types and Incidence
A larva with a normal set of chemosensilla and larvae with a missing or deformed epipharynx, antenna, maxilla, maxillary palp, and styloliconica were selected for SEMs (Fig. 1). An experiment to determine the incidence of these deformities consisted of 10 replicate trials of rearing larvae in four population densities (10, 40, 60, and 100 per box). In the first trial, we recorded that a total of 75 of the 196 surviving larvae across all four boxes had missing or malformed chemoreceptor organs. In the subsequent nine trials (1,744 surviving larvae, 522 of which were deformed), we recorded not only the presence of the deformity, but the type of deformity as well (Fig. 2). Some larvae (~10%) had more than one type (‘Multiple’ in Figs. 2, 4, and 6). We did not find a significant association between types of deformities and population density (Fig. 2, P = 0.3747, chi-squared test of independence).

Effect of Population Density on Incidence of Chemosensory Deformities and Mortality
The incidence of deformities (% of larvae deformed) increased linearly with population density (Fig. 3A; Table 1, A), ranging from 10% in the least dense cohorts of 10 larvae/box, to 39.9% in the highest density cohorts of 100 larvae/box. On average, the incidence of deformed animals increased by 3% for every 10-animal increase in cohort size. Mortality was zero in the lowest density cohort but increased to ~5% in the three higher-density cohorts (Fig. 3A; Table 1, B).

The question arises whether volatile chemicals (e.g., hypothetical stress pheromones, products of metabolism, etc.) emitted by crowded larvae may have affected the development of other larvae in their culture box. To test this, we compared the incidence of deformities of larvae reared in a high population density on a single platform versus the same number of larvae at low density in a tier of three platforms. Results showed that the incidence of deformities was significantly lower on the low-density tier platforms than on the high-density single platforms (Fig. 3B; Table 2, A). (There was no significant difference among tier platforms themselves, Table 2, B.) Thus, area-associated factors, such as physical contact between...
larvae, likely played more of a role in causing deformities than volume-associated factors, such as volatile chemicals.

**Effect of Population Density and Chemosensory Deformities on Larval Mass**

Larval mass decreased as population density increased; this was true for both normal and deformed larvae, as well as for the combined effects of deformities and population density (Fig. 4A; Table 3). Moreover, larval mass varied with chemoreceptor deformity status (i.e., normal and types of deformities) (Table 4, A), and larvae with specific deformities of maxilla, palp, or multiple all had significantly lower mass than normal larvae (Fig. 4B; Table 4, B).

**Effect of Population Density on Larval Food Consumption**

The average daily food consumption per larva changed significantly during days 5–10 of the culture (Fig. 5). Two effects are clearly visible. First, consumption increased early in this period as larvae molted into the fourth instar and began feeding; consumption peaked by days 7–8 and then decreased as larvae stopped feeding at the end of the fourth instar. These are normal feeding dynamics of larval cultures, and thus food consumption regressed onto day of culture is statistically significant as expected (Fig. 5; Table 5, positive slope). The other effect, one that is more relevant to this study, is that food consumption decreased as population density increased (Fig. 5; Table 5, negative slope).

**Effect of Population Density and Deformities on Duration of Development**

The number of larvae in each cohort remained nearly constant until day 7 when the most rapidly developing larvae reached the end of their fourth instar and were removed as premolts (Fig. 6A). Population curves of higher-density cohorts are right-shifted towards later times, as they took longer to develop; this was true for both normal and deformed larvae (Fig. 6A; Table 6, A). ANOVA found significant variation in duration of development across deformity status categories (Fig. 6B; Table 6, B), and further analysis showed that larvae with deformities of the maxilla or maxillary palp had longer development times than normal larvae (Fig. 6B; Table 6, C).

---

**Table 1. Effect of population density on incidence of deformities and mortality**

| Cohort Density |\( b_0 \) | SE (\( b_0 \)) | \( b_1 \) | SE (\( b_1 \)) | \( SE_{\text{Total}} \) | \( F \) | df | \( P \) | \( R^2_{\text{adj}} \) |
|----------------|---------|---------------|---------|---------------|----------------|--------|-----|-------|-------------|
| 10             | 0.0670  | 0.0287        | 0.0036  | 0.0005        | 0.0945         | 51     | 1,38 | <0.0001 | 0.5626      |
| 372            |         |               |         |               |                |        |      |        |             |
| 565            |         |               |         |               |                |        |      |        |             |
| 906            |         |               |         |               |                |        |      |        |             |

Statistical parameters for Fig. 3A. Linear regression: (A) Model: \( y = b_0 + b_1 x_1 \), where \( y \) = incidence of deformities (% larvae deformed), \( b_0 \) = intercept, \( b_1 \) = slope, \( x_1 \) = population density; 1,744 larvae. (B) Model: \( y = b_0 + b_1 x_1 \), where \( y \) = incidence of mortality; 93 deaths.
Discussion

In our experience with *M. sexta* larvae reared in isolation, chemosensory deformities were rarely seen. In larvae from group cultures, however, such deformities occurred frequently, and experiments showed that the incidence of missing or deformed chemosensilla was directly proportional to population density. Our data also showed that three life history traits (larval mass, food consumption, and development rates) decreased with increases in population density for both normal larvae and those with chemosensory deformities. These effects are similar to those of certain other lepidopterans, such as the speckled wood butterfly *Pararge aegeria* (Gibbs et al. 2004) and the silkworm *Bombyx mori* (Dutta et al. 2013). Interestingly, the opposite effects were seen in larvae of the Glanville fritillary butterfly *Melitaea cinxia* which grew faster and larger when reared in high-density conditions (Rosa et al. 2017), as did larvae of the gypsy moth *Lymantria dispar* (Pavlushin et al. 2019) and the moth *Cnaphalocrocis medinalis* (Yang et al. 2015). Thus, crowding effects on life history traits appear to be common phenomena, but we are unaware of any density-related occurrences of missing or deformed chemosensilla in other species.

The results of our experiments with *M. sexta* raise questions about the mechanisms responsible for these findings. First, what aspects of larval density might have led to these results? One possibility is that stressors resulting from crowding caused changes of internal chemical factors that modified normal developmental processes. In the gypsy moth *Lymantria dispar*, for example, larval crowding results in a decreased concentration of the hormone dopamine in the hemolymph (Pavlushin et al. 2019). In *Drosophila*, crowding induces heat shock proteins (reviewed by King and MacRae 2015) and affects the dopamine/ecdysteroid receptor that modulates intracellular signaling pathways in response to various stressors (reviewed by Petruccelli et al. 2020). In locusts, higher population density causes solitary-to-gregarious phase transformation, and many internal regulatory chemical agents and signal molecules are known to play a role (reviewed by Ayali 2019).

Secondly, could external chemical factors, either volatile or non-volatile, have played a role in chemosensilla alterations? There are examples of this in locusts: volatiles from frass and body odors affect the transition from solitary-to-gregarious phase (Heifetz et al. 1996, Wei et al. 2017, Ayali 2019). However, in *M. sexta*,

---

### Table 2. Tier experiment: different area densities but constant volume density

| Variable 1 | Incidence | SE | n | Variable 2 | Incidence | SE | n | Test statistic | P |
|------------|-----------|----|---|------------|-----------|----|---|----------------|---|
| Incidence, tier platforms | 11.28 | 2.31 | 461 | Incidence, single platforms | 22.73 | 4.01 | 164 | t = 2.491 | 0.032 |

(A) Statistical parameters for Fig. 3B. The t-test was used to compare incidence of deformities in larvae reared on tier platforms vs. single platforms. (B) ANOVA compared the incidence of deformities across cultures reared on the tier platforms. n = number of larvae.

### Fig. 4. Larval mass varies with population density and chemosensory deformity. (A) Average mass (g ± SE) of larvae in each cohort. n = the number of normal (square symbols) or deformed (round symbols) larvae in the designated cohort summed over nine trials. The SE of some cohorts is smaller than the symbols. Statistical parameters are in Table 3. (B) Average mass (g ± SE) of normal and deformed larvae, and for each type of deformity: Max = maxilla, Palp = maxillary palp, Ant = antenna, Epi = epipharynx, Styl = maxillary styloconica, Multi = multiple chemosensory deformities. n = total number of larvae in the indicated category summed over nine trials. Statistical parameters are in Table 4.
Table 3. Effect of population density and chemosensory deformities on larval mass

| Variable 1 | n  | Category | No. of categories | Test statistic | df | P  | R² adj
|------------|----|----------|-------------------|----------------|----|----|--------
| Mass       | 1,734 | Deformity status | 7 | F = 7.298 | 6,1726 | <0.0001 |
| Mass, normal | 1,217 | Mass, maxilla deformity | 192 | -0.0898 | <0.0001 |
| Mass, normal | 1,217 | Mass, palp deformity | 189 | -0.0594 | 0.0198 |
| Mass, normal | 1,217 | Mass, multiple deformities | 55 | -0.1117 | 0.0095 |

Statistical parameters for Fig. 4A. Linear regressions: (A) Model: $y = b_0 + b_1x_1 + b_2x_2$, where $y$ = mass (g), $b_0$ = intercept, $b_1$ = slope, $x_1$ = population density. (B) Model: $y = b_0 + b_1x_1 + b_2x_2$, where $b_2$ = slope, $x_2$ = indicator of presence of a deformity (0 = normal, 1 = deformed); $n$ = 1,217 normal and 517 deformed larvae in nine trials.

Table 4. Effects of specific deformities on larval mass

| Variable 1   | n  | Variable 2            | n  | Difference between means (g) | P    |
|--------------|----|-----------------------|----|-------------------------------|------|
| Mass, normal | 1,217 | Mass, maxilla deformity | 192 | -0.0898                      | <0.0001 |
| Mass, normal | 1,217 | Mass, palp deformity   | 189 | -0.0594                      | 0.0198 |
| Mass, normal | 1,217 | Mass, multiple deformities | 55  | -0.1117                      | 0.0095 |

Thirdly, could physical factors have been responsible for the effects of larval crowding? Larva-to-larva contact resulting in injury might have occurred in crowded conditions, so some chemosensilla may have been damaged during feeding in the third and fourth instars. Our data were obtained after the molt into the fifth-instar larvae, so if damage had occurred earlier, the sensory primordial tissues should have formed normal new chemosensilla during the quiescent premolt period. However, deformations were very much in evidence (Fig. 3A), so the appropriate primordial tissues must have been missing, damaged, or not activated by growth or molting hormones.

Another physical factor could be contact disturbance during feeding. This may have been responsible for the reduced consumption seen in the higher-density cohorts (Fig. 5). In general, when rates of consumption drop below the rates of protein absorption and utilization, the result is a reduction in nutrition (Woods and Kingsolver 1999). Low nutrition is known to affect insect development in many ways. For one example, three different studies on dipterans showed that larval life history traits were significantly lower for larvae reared in high densities. However, these effects were only seen when diet was limiting. When diet was not limiting, life history traits were normal (Gilles et al. 2011, Alto et al. 2012, Klepsatel et al. 2018).

Thus, low nutrition, not crowding, appears to have caused these changes in life history traits.

For low nutrition to affect growth rates, larvae must be able to detect nutrient concentrations. In Drosophila, there are two sensing systems, one for amino acids (Colombani et al. 2003) and another for carbohydrates and lipids (Ikeya et al. 2002). The latter system is been shown to play a role in chemical regulation of phase change (Heifetz et al. 1996, 1998).

Statistical parameters for Fig. 4B. (A) ANOVA was used to compare larval mass (g) across all categories of deformity status, including normal and six types of specific deformities. (B) ANOVA with Tukey's multiple comparisons of means was used to compare the mean mass of normal larvae vs. that of larvae having the indicated deformity. n = number of animals weighed in nine trials.
comprised of neurosecretory cells in the brain that secrete insulin-like peptides which control the growth of peripheral tissues. The two systems are hormonally linked, so any or all of these three categories of nutrients can control growth of both peripheral and primordial imaginal tissues (Koyama and Mirth 2016; reviewed by Koyama and Mirth 2018). *Manduca sexta* likely has a similar system; in low nutrition situations, it would reduce body growth and other life history traits and slow the growth of primordial tissues, presumably including those for chemosensilla which may not develop if not stimulated enough. This coordinated growth control might also

![Image](image-url)
explain why the incidence of chemosensilla deformations is strongly correlated with lower mass and rate of development (Figs. 4 and 6).

In addition to the above discussion of possible mechanisms, we are also mindful of feeding behavior and whether it could be affected by the loss of one or a few chemosensilla. Reports in the literature (de Boer 1991, Flowers and Yamamoto 1992) show that the loss of half of the set of oral chemosensilla via unilateral surgical ablations resulted in some loss of food plant discrimination. Presumably, a similar behavioral deficit would be observed in larvae with the chemosensilla loss or deformity found in our experiments. The question then arises whether this could ever benefit the insect. Consider situations where oligophagous larvae in crowded rearing conditions must emigrate from a host plant that has been mostly consumed; could larvae possessing fewer functional chemoreceptors now accept some non-host plants, thereby avoiding dehydration or starvation while searching for their normal hosts? One might speculate that this could be a mechanism that evolved to loosen the tight food-choice constraints of mono- or oligophagous in situations of high population densities.

In summary, the experimental results presented here expand our understanding of the effects of larval crowding by reporting new information on another species, *M. sexta*, and on a new set of target organs, the chemosensilla. Crowding resulted in a decrease in three life history traits similar to that seen in several other species. In addition, crowding increased the incidence of missing and deformed chemosensilla, an effect which, to our knowledge, had not been previously reported. We considered possible causes and mechanisms for these effects based on research on other species, but the definitive data for *M. sexta* await future research.

**Acknowledgments**

The authors thank Haixin Xu for the electron microscopy and the UMBC Physics Department for use of its scanning electron microscope; Christopher Wells, Jacki Gutenkunst, and Ian Hall for assistance with cultures and experiments; and also Tim Ford, Christopher Wells, and Jeremy Swan for the figures.

**References Cited**

Alto, B. W., E. J. Muturi, and R. I. Lampman. 2012. Effects of nutrition and density in *Culex pipiens*. Med. Vet. Entomol. 26: 396–406.

Ayali, A. 2019. The puzzle of locust density-dependent phase polyphenism. Curr. Opin. Insect Sci. 35: 41–47.

Colombani, J., S. Raisin, S. Pantalacci, T. Radimerski, J. Montagne, and P. Léopold. 2003. A nutrient sensor mechanism controls *Drosophila* growth. Cell 114: 739–749.

de Boer, G. 1991. Role of bilateral chemosensory input in food discrimination by *Manduca sexta* larvae. Entomol. Exp. Appl. 61: 159–168.

de Boer, G. 2006. The role of the antennae and maxillary palps in mediating food preference by larvae of the tobacco hornworm, *Manduca sexta*. Entomol. Exp. Appl. 119: 29–38.

de Boer, G., and F. E. Hanson. 1987. Differentiation of roles of chemosensory organs in food discrimination among host and non-host plants by larvae of the tobacco hornworm, *Manduca sexta*. Physiol. Entomol. 12: 387–398.

Dutta, S., M. Deka, and D. Devi. 2013. Impact of crowding on larval traits and silk synthesis in *eri* silkworm. Entomol. News 123: 49–58.

Flowers, R. W., and R. T. Yamamoto. 1992. Feeding on non-host plants by partially maxillectomized tobacco hornworms (*Manduca sexta*: Lepidoptera: Sphingidae). Fla. Entomol. 75: 89–93.

Gibbs, M., L. A. Lace, M. J. Jones, and A. J. Moore. 2004. Intraspecific competition in the speckled wood butterfly *Pararge aegeria*: effect of rearing density and gender on larval life history. J. Insect Sci. 4: 16.

Gilles, J. R., R. S. Lees, S. M. Soliban, and M. Q. Benedict. 2011. Density-dependent effects in experimental larval populations of *Anopheles arabiensis* (Diptera: Culicidae) can be negative, neutral, or overcompensatory depending on density and diet levels. J. Med. Entomol. 48: 296–304.

Glendinning, J. I., C. Foley, I. Loncar, and M. Rai. 2009. Induced preference for host plant chemicals in the tobacco hornworm: contribution of olfaction and taste. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 195: 591–601.

Hanson, F. E., and V. G. Dehier. 1973. Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 19: 1019–1034.

Heifetz, Y., H. Voet, and S. W. Applebaum. 1996. Factors affecting behavioral phase transition in the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). J. Chem. Ecol. 22: 1717–1734.

Heifetz, Y., I. Miloslavski, Z. Aizenstat, and S. W. Applebaum. 1998. Cuticular surface hydrocarbons of desert locust nymphs, *Schistocerca gregaria*, and their effect on phase behavior. J. Chem. Ecol. 24: 1033–1047.

Ikeya, T., M. Galactic, P. Belawat, K. Naiz, and E. Hafen. 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. Curr. Biol. 12: 1293–1300.

King, A. M., and T. H. MacRae. 2015. Insect heat shock proteins during stress and diapause. Annu. Rev. Entomol. 60: 59–75.

Klepatsel, P. E., Procházka, and M. Gálková. 2018. Crowding of *Drosophila* larvae affects lifespan and other life-history traits via reduced availability of dietary yeast. Exp. Gerontol. 110: 298–308.

Koyama, T., and C. K. Mirth. 2016. Correction: growth-blocking peptides as nutrition-sensitive signals for insulin secretion and body size regulation. PLoS Biol. 14: e1002551.

Koyama, T., and C. K. Mirth. 2018. Unravelling the diversity of mechanisms through which nutrition regulates body size in insects. Curr. Opin. Insect Sci. 25: 1–8.

Moore, M. E., K. M. Kester, and J. G. Kingsolver. 2020. Rearing temperature and parasitoid load determine host and parasitoid performance in *Manduca sexta* and *Cotesia congregata*. Ecol. Entomol. 45: 79–89.

Pavlushin, S. V., I. A. Belousova, E. A. Chertlova, N. A. Kryukova, V. V. Glupov, and V. V. Martemyanov. 2019. The effect of population density of *Lymantria dispar* (Lepidoptera: Erebidae) on its fitness, physiology and activation of the covert nucleopolyhedrovirus. Eur. J. Entomol. 116: 85–91.

Petrucelli, E., A. Lark, A. Mirkvicka, and T. Kitamoto. 2020. Significance of DopECr, a G-protein coupled dopamine/ecdysiester receptor, in physiological and behavioral response to stressors. J. Neurogenet. 34: 55–68.

Rosa, E., S. van Noubuys, and M. Saastamoinen. 2017. The more the merrier: conspecific density improves performance of gregarious larvae and reduces susceptibility to a pupal parasitoid. Ecol. Evol. 7: 10710–10720.

Rowley, M., and F. Hanson. 2007. Humidity detection and hygropreference behavior in larvae of the tobacco hornworm, *Manduca sexta*. J. Insect Sci. 7: 1–10.

Stådler, E., and F. E. Hanson. 1975. Olfactory capabilities of the ‘gustatory’ chemoreceptors of the tobacco hornworm larvae. J. Comp. Physiol. 104: 97–102.

Waldbaur, G. P., and G. Fraenkel. 1961. Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm, *Protoparce sexta* (Lepidoptera: Sphingidae). Ann. Entomol. Soc. Am. 54: 477–485.

Wei, J. N., B. W. Shao, X. H. Wang, J. Ge, X. Chen, D. Yu, and L. Kang. 2017. Composition and emission dynamics of migratory locust volatiles in response to changes in developmental stages and population density. Insect Sci. 24: 60–72.

Woods, H. A., and J. G. Kingsolver. 1999. Feeding rate and the structure of protein digestion and absorption in lepidopteran midguts. Arch. Insect Biochem. Physiol. 42: 74–87.

Yamamoto, R. T. 1969. Mass rearing of the tobacco hornworm, *Manduca sexta*. II. Larval rearing and pupation. J. Econ. Entomol. 62: 1427–1431.

Yang, F., G. Hu, J. J. Shi, and B. P. Zhao. 2015. Effects of larval density and food stress on life-history traits of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). J. Appl. Entomol. 139: 370–380.