

Increased Mortality of Isolated First Instar Larvae of *Inachis io* (Lepidoptera)

É. LAUBER* and B. DARVAS

Plant Protection Institute, Hungarian Academy of Sciences,
P.O. Box 102, H-1525 Budapest, Hungary

(Received: 25 September 2008; accepted: 2 December 2008)

The European peacock butterfly, *Inachis io* Linnaeus is one of those lepidopteran species whose larvae stay together after hatching. Larvae usually aggregate in batches of hundreds. Isolated first instar larvae, although spin or feed to some extent, spend most of their time apparently waiting for a signal yet unknown for continuation, and finally end up dying until the second moult. In our experiments the mortality of isolated larvae varied significantly from 25% to 75%, indicating that loneliness induces a kind of death signal of unknown origin in first instar larvae of *I. io*.

Keywords: *Inachis io*, isolated first instar larvae, death signal.

The European peacock butterfly, *Inachis io* Linnaeus (Lepidoptera: Nymphalidae) is one of the numerous protected butterfly species in Hungary where two generations grow up in a year. Females lay eggs in pyramid-shaped batches of 100–300 on the back side of the leaf of stinging nettle, *Urtica dioica* Linnaeus. After hatching, the larvae feed together on leaves and shoots, until at least the 4th instar.

In eusocial species individuals cooperate in brood care, reproductive functions are divided, and generations overlap with one another (Barrows, 2001). However, sociality must be defined much more broadly on the grounds of communication. Wasps, bees and ants are capable of extensive communication, while communication of Lepidoptera species is usually restricted to group cohesion involves mostly tactile or olfactoric signals. Nevertheless, in some cases lepidopteran larvae show cooperation as sophisticated in pheromonal foraging and recruitment trails as eusocial species. Moving from low to high along the continuum of social complexity, weakly or facultative social species show patch-restricted foraging with group defense only. The greatest number of social lepidopteran species exhibit nomadic foraging: they communicate chemically or visually for defense and cohesion (and in some cases local orientation to food). Beyond that, the most complex and sophisticated lepidopteran societies have the ability to communicate the location of food (Costa and Pierce, 1997).

* Corresponding author; e-mail: eva.lauber@gmail.com

Materials and Methods

Batches of peacock eggs were collected from our breeding based on collection in Adyliget, 2002. In this experiment freshly hatched first instar larvae were used, 50 isolated and 50–50 grouped (10 groups of five and 5 groups of 10). All larvae used in a single experiment originated from the same batch. Since a female usually copulate with only one male (Wiklund et al., 2003), the offspring was considered genetically uniform. Four experiments were maintained: one at 21 ± 1 °C, one at 25 ± 1 °C, and two at 26 ± 1 °C. In all cases, caterpillars were fed with the leaves of stinging nettle. Completely expanded leaves of the 5th to 8th tiers from top were used. Each leaf was placed into a separate laboratory glass, and the approximately 3–6 hour-old first instar larvae were placed on them. Larvae were carefully manipulated with a fine brush under microscope to avoid damage. In case of groups, larvae were placed close to each other, following their natural gregarious manner, within a 1 cm² surface area near the centre of the leaf. Laboratory experiments were maintained at a long photoperiod (light 14 : dark 10). All data were analyzed with Statistica[®] software.

Results

Larvae in groups of ten wandered on the leaf together, and then they started feeding within a short time after having been placed there. All the time they remained together, with few exceptions. Larvae in groups of five often left each other, and dispersed on the leaf. Unless they found each other, they died alone. Sometimes they divided into two groups on a leaf. This, however, also enhanced their mortality. Isolated larvae spent little time wandering, then most of them waited in a stationary manner for a long period. A part of them just sat at place until death, whilst others started feeding. However, eating was not sufficient for survival, as some of them gave up it in 1 or 2 days and then died. Most of the isolated larvae did not even start feeding. The mortality of the isolated larvae was always significantly greater than that of those reared in groups of 5 or 10 (*Figs 1, 2, 3, 4*). It seems that the rate of death depended not only on the number of individuals, but on the temperature.

At 21 °C (*Fig. 1*) the mortality of isolated larvae was approximately 25%, while at 25–26 °C it rose up to 65–75% on average (*Figs 2, 3, 4*). The mortality of larvae reared in groups of five varied depending on their dispersal. Spreading out isolated them and raised the number of deaths (*Figs 2 and 4*). In the third experiment the average mortality was 10% in groups of five, similarly to groups of ten. In contrast, in the fourth experiment, carried out as a repetition of the third, there appeared a significantly greater, 45% average mortality among groups of five (*Figs 3 and 4*). Both of these experiments were conducted on 26 °C, so this altering mortality does not depend on temperature. However, death rates rose with increasing temperature, except in groups of ten where on average 92–98% of the larvae survived.

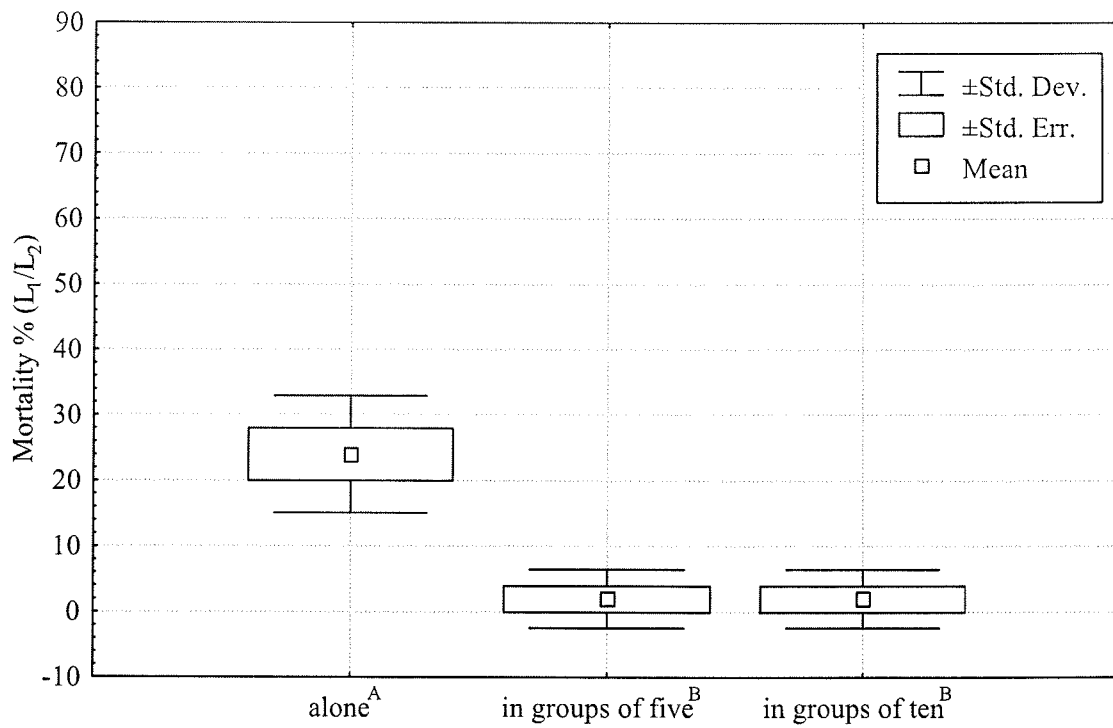


Fig. 1. Mortality rates of *Inachis io* larvae reared in different size groups at 21 °C. Means within a column followed by the same capital letter are not significantly different ($SD_{1\%}$: 12%)

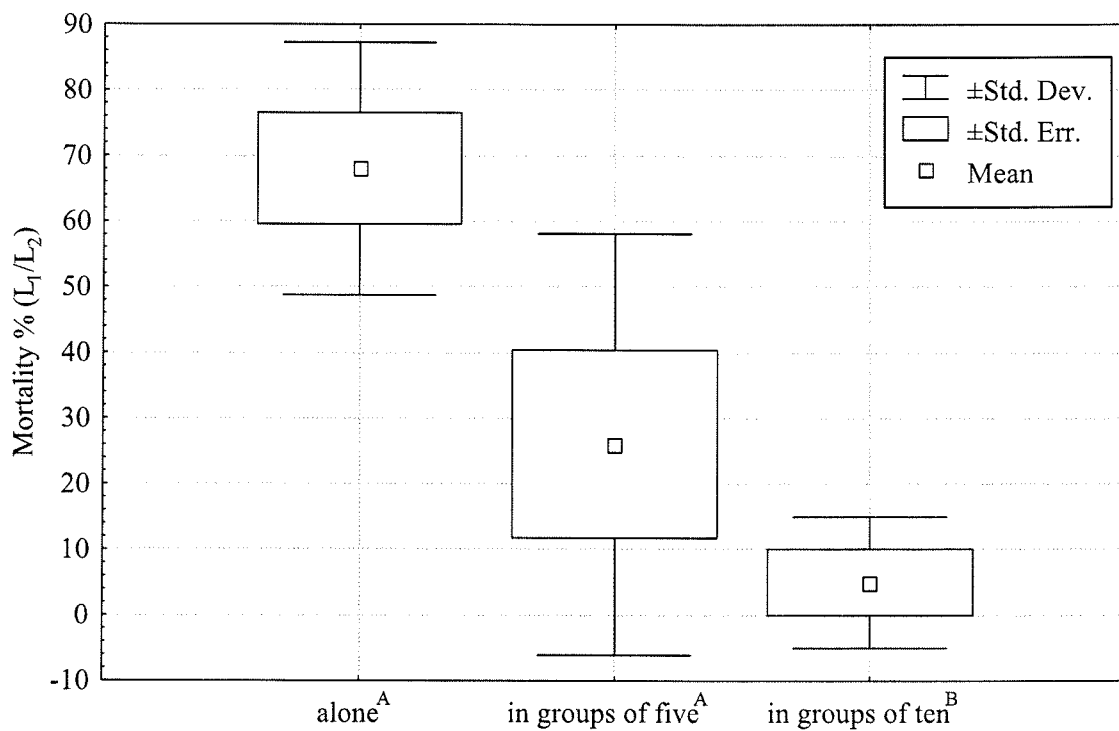


Fig. 2. Mortality rates of *Inachis io* larvae reared in different size groups at 25 °C. Means within a column followed by the same capital letter are not significantly different ($SD_{1\%}$: 44%)

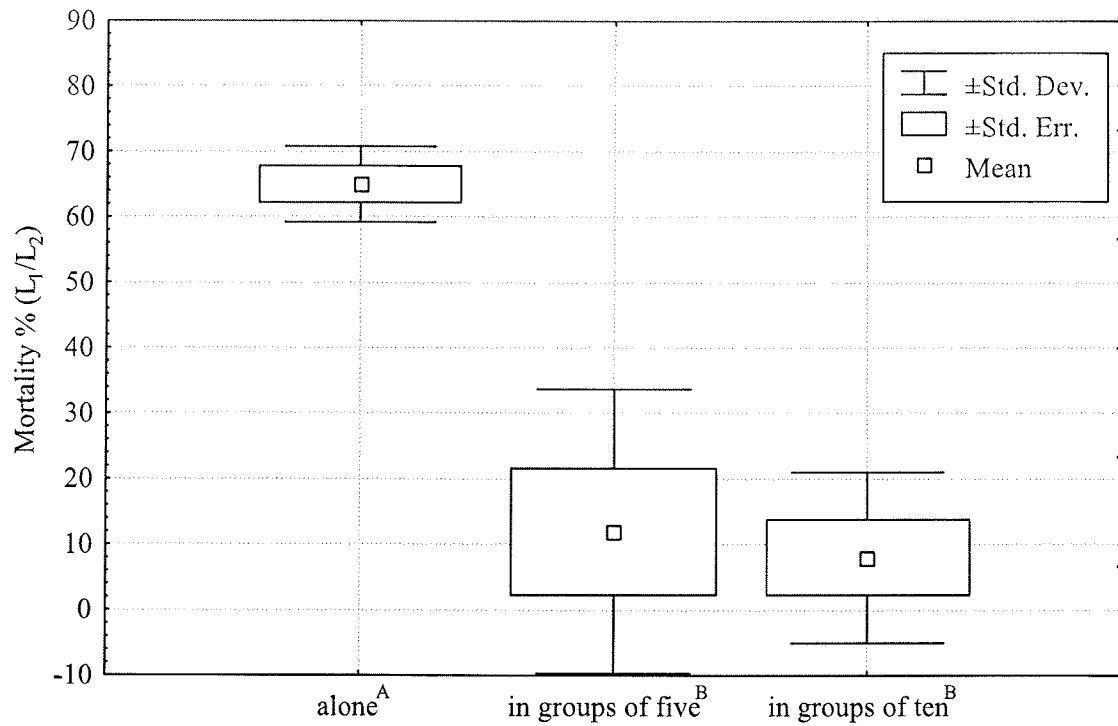


Fig. 3. Mortality rates of *Inachis io* larvae reared in different size groups at 26 °C. Means within a column followed by the same capital letter are not significantly different ($SD_{1\%}$: 30%)

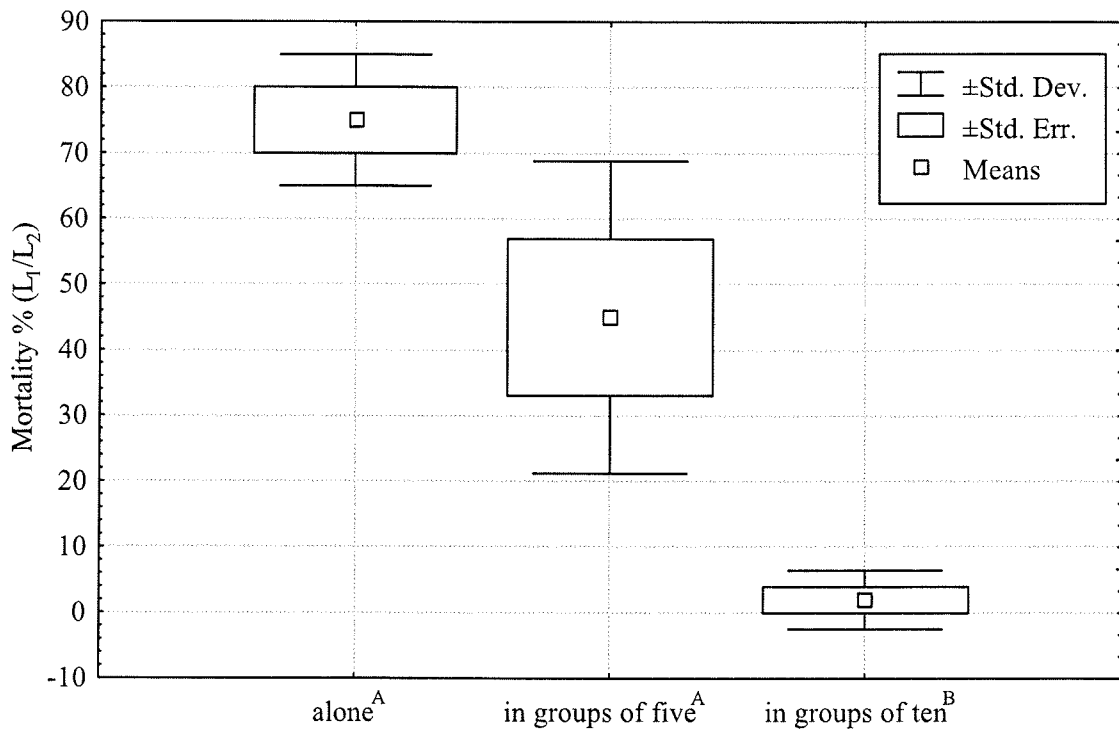


Fig. 4. Mortality rates of *Inachis io* larvae reared in different size groups at 26 °C. Means within a column followed by the same capital letter are not significantly different ($SD_{1\%}$: 28%)

Discussion

Certain butterfly species – living on nettle – lay a single egg at a time, and the larva starts to feed and spin alone (e.g. the leaf-rolling larva of red admiral, *Vanessa atalanta* Linnaeus) or in pairs (e.g. the peeler larvae of comma butterfly, *Polygonia c-album* Linnaeus). Other species' larvae derive from a bunch of eggs and quickly disperse after hatching. For example, larvae of the European corn borer (*Ostrinia nubilalis* Hübner) spread out after hatching, and their dispersal is strengthened by meeting with siblings. Cannibalistic behaviour of larvae is known in this case. Opposite of this, newly hatched peacock larvae usually aggregate in batches of hundreds, which can be enhanced by synchronous hatching of eggs.

There are several theories why mortality increases in case of lonely *I. io* larva:

(1) Genetic background – In our experiments different results were found in the case of different egg batches. This suggests an inherited mechanism of handling loneliness.

(2) Abiotic environment – Temperature, humidity and photoperiodism are usually mentioned as factors that can modify the development of larvae via the endocrine system. Thermoregulation can also be obtained by basking or collective metabolic heat production. The latter characterizes the tent-building caterpillars (Ruf and Fiedler, 2000), or even some mass-forming (80–120 individuals) caterpillars like *Aglais urticae* Linnaeus and *I. io* (Mosebach-Pukowski, 1938). Heat-gain is heightened with group size, while the temperature of solitary larvae is near the ambient temperature. When temperatures rise and relative humidity decreases, *I. io* larvae protect themselves by gathering tightly together (Willmer, 1980). In inverse proportion to the relative humidity (RH), dehydration of isolated larvae increases, and the haemolymph osmolality of solitary larvae, as a function of RH, rises steeper than that for larvae in groups (Willmer, 1980). This might increase the mortality rates.

(3) Biotic environment (early engrams, stress and immune system) – In some lepidopteran species and many other insects (aphids, locusts and planthoppers), density-dependent phase polyphenism is a known phenomenon (Applebaum and Heifetz, 1999). It is induced by, among other things, rearing density, and it has a profound effect on larval physiology, morphology, behaviour, development rates, metabolism, etc. (Kunimi and Yamada, 1990; Darvas and Polgár, 1994). It seems that the density-dependent physiological phase and prophylaxis are attached by means of the phenoloxidase enzyme system, which is also involved in immune response and melanization (Reeson et al., 1998; Sugumaran, 2002). *Spodoptera exempta* Walker larvae reared at a high density were found to be considerably more resistant to a nuclear polyhedrosis virus than those reared in isolation (Reeson et al., 1998). Similar decreasing susceptibility with increasing rearing density has been described in *Pseudaletia separata* Walker (Kunimi and Yamada, 1990). Individually reared larvae were ca. 2, 8 and 10 times more susceptible to nuclear polyhedrosis virus (PsNPV) than larvae reared at densities of 2, 5 and 20 per container, respectively. European populations of *I. io* larvae are frequently infected by cyrovirus 2. This CPV type 2 (Payne and Tinsley, 1974) mainly cause death at late instars. However, it can develop earlier if the immune system is weakened by any reason.

(4) Semiochemical communication – Trail-tracking of gregarious caterpillars (belonging to the families of Lasiocampidae, Nymphalidae, Pieridae, Saturniidae, Tortricidae) can be mediated by silk and/or pheromones (Costa and Pierce, 1997; Fitzgerald and Underwood, 1998; Ruf et al., 2001). *I. io* larvae start spinning immediately after hatching. They cover the batch of eggs' shells and the leaf with fine silk. After they consume all the leaf, except its glandular hairs, they crawl along and spin continuously. Siblings follow each other by silk trails.

Rapid death is known among isolated individuals of eusocial bees or ants. In honey bee, *Apis mellifera* Linnaeus, when hive bees develop into foragers, their juvenile hormone (JH) titer increases, causing a cessation of their vitellogenin production (Amdam et al., 2003). The synthesis of food-processing enzymes lowers as well, so foragers are not able to digest pollen. The rise in JH titer associated with the hive bee to forager transition causes apoptosis of the hemocyte population in the forager haemolymph (Amdam et al., 2004). So the foragers are at the mercy of their hives, who feed them with proteinaceous royal jelly containing antibacterial compounds (Amdam et al., 2003; 2004). The hive bee to forager transition is a rapid process caused by isolation (losing the olfactory and tactile contacts).

The phenomenon of loneliness-induced mortality in a species, *I. io*, not known as a social insect, moreover, death rate being dependent on group size of the hatching larvae, temperature and possibly other environmental factors certainly prompts the elucidation of its biochemical background: possible hormonal or pheromonal interactions that may trigger such response. Further studies in this area are going on.

Literature

- Amdam, G. V., Norberg, K., Hagen, A. and Omholt, S. W. (2003): Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. USA* 100, 1799–1802.
- Amdam, G. V., Simoes, Z. L. P., Hagen, A., Norberg, K., Schroder, K., Mikkelsen, O., Kirkwood, T. B. L. and Omholt, S. W. (2004): Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. *Exp. Geront.* 39, 767–773.
- Applebaum, S. W. and Heifetz, Y. (1999): Density-dependent physiological phase in insects. *Ann. Rev. Entomol.* 44, 317–341.
- Barrows, E. M. (2001): *Animal Behavior Desk Reference; a dictionary of animal behavior, ecology, and evolution.* CRC Press LLC, pp. 1–922.
- Costa, J. T. and Pierce, N. E. (1997): Social evolution in the Lepidoptera: Ecological context and communication in larval societies. In: J. C. Choe and B. J. Crespi (eds): *The Evolution of Social Behavior in Insects and Arachnids.* Cambridge University Press, Cambridge, UK, pp. 407–442.
- Darvas, B. and Polgár, A. L. (1994): Ízeltlábúak alkalmazkodási stratégiái. I. *Sensu lato* polimorfizmus: polifenizmus. [Adaptive strategies in Arthropod species. I. Polymorphism *sensu lato*: polyphenism.] *Növényvédelem* 30, 353–361.
- Fitzgerald, T. D. and Underwood, D. L. A. (1998): Trail marking by the larva of the madrone butterfly *Eucheira socialis* and the role of the trail pheromone in communal foraging behavior. *J. Insect Behav.* 11, 247–263.
- Kunimi, Y. and Yamada, E. (1990): Relationship of larval phase and susceptibility of the armyworm, *Pseudaletia separata* Walker (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus and a granulosis virus. *Appl. Ent. Zool.* 25, 289–297.
- Mosebach-Pukowski, E. (1938): Über die Raupengesellschaften von *Vanessa io* und *Vanessa urticae*. *Z. Morphol. Ökol. Tiere* 33, 358–380.

- Payne, C. C. and Tinsley, T. W. (1974): The structural proteins and RNA components of a cytoplasmic polyhedrosis virus from *Nymphalis io* (Lepidoptera: Nymphalidae). *J. Gen. Virol.* 25, 291–302.
- Reeson, A. F., Wilson, K., Gunn, A., Hails, R. S. and Goulson, D. (1998): Baculovirus resistance in the noctuid *Spodoptera exempta* is phenotypically plastic and responds to population density. *Proc. R. Soc. London* 265, 1787–1791.
- Ruf, C. and Fiedler, K. (2000): Thermal gains through collective metabolic heat production in social caterpillars of *Eriogaster lanestris*. *Naturwissenschaften* 87, 193–196.
- Ruf, C., Costa, J. T. and Fiedler, K. (2001): Trail-based communication in social caterpillars of *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). *J. Insect Behav.* 14, 231–245.
- Sugumaran, M. (2002): Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Res.* 15, 2–9.
- Wiklund, C., Gotthard, K. and Nylin, S. (2003): Mating system and the evolution of sex-specific mortality rates in two nymphalid butterflies. *Proc. R. Soc. London* 270, 1823–1828.
- Willmer, P. G. (1980): The effects of fluctuating environment on the water relations of larval Lepidoptera. *Ecol. Entomol.* 5, 271–292.