Journal of Insect Physiology 57 (2011) 682–687

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Phenotypic plasticity in the reproductive traits of a parasitoid

Véronique Martel [a,b,*], Éric Darrouzet [c], Guy Boivin [a]

[a] Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Boul. Gouin, St-Jean-sur-Richelieu, Québec, Canada J3B 3E6
[b] Department of Natural Resource Sciences, Macdonald Campus, McGill University, 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec, Canada H9X 1C0
[c] Université de Tours, Faculté des Sciences, Institut de Recherche sur la Biologie de l'Insecte (IRBI), UMR CNRS 6035, Parc de Grandmont, 37200 Tours, France

ARTICLE INFO

Article history:
Received 30 November 2010
Received in revised form 18 January 2011
Accepted 19 January 2011

Keywords:
Size
Sperm production
Sperm storage
Oocyte
Gamete size
Nutritional quality
Trichogramma euproctidis

ABSTRACT

Organisms show phenotypic plasticity – the capacity for a given genotype to express different phenotypes – in response to changes in the environment. Among the several factors that can cause phenotypic plasticity, nutritional constraints during development can affect the size of organisms and, consequently, affect most life-history traits, including reproductive traits. As their larvae are restricted by the amount of food contained in their host, parasitoids are a good model to study phenotypic plasticity related to size. The phenotypic plasticity of reproductive traits was investigated in the egg parasitoid *Trichogramma euproctidis* (Hymenoptera: Trichogrammatidae) by using host species of different sizes. Adult size, sperm storage organs (seminal vesicles and spermatheca), number of sperm stored and gamete size (sperm and oocyte) are all influenced by the host species; larger individuals have larger organs which contain more sperm, and both sperm and oocytes are correlated with adult size. However, while females become larger than males and mature larger oocytes in larger hosts, increase in sperm length stops after a given threshold.

1. Introduction

The environment in which organisms live varies, both spatially and temporally, according to biotic (competition, predation, etc.) and abiotic (temperature, humidity, etc.) factors. In order to perform well in this variable environment, organisms often show flexibility in the expression of a character: this flexibility is called phenotypic plasticity. Phenotypic plasticity is the capacity of a single genotype to produce different phenotypes depending on the environmental conditions (Roff, 2002). It can affect most life history traits including physiology and behaviour. These changes can express themselves rapidly on short term, for example when a behaviour is modified in response to a given cue (Gorur et al., 2007; Kause et al., 1999), or take place on long term, for example during development, and affect the adult permanently (Brakefield et al., 1996; Dufty et al., 2002). Phenotypic plasticity has an important role in maximizing fitness in a new environment.

Even if phenotypic plasticity can be adaptive by allowing for the most optimal trait to be expressed in a given environment, it can also result from constraints. In such cases, phenotypic plasticity can permit an individual to complete its development under adverse conditions at the cost of a smaller size for example (Blanckenhorn, 1998).

The size of an animal is determined by its genotype modified by the phenotypic plasticity that depends on the developmental conditions, such as climate (reviewed by Atkinson, 1994) or availability and quality of food. This has been shown for many animals such as mammals (Widdowson and Kennedy, 1962; Widdowson and McCance, 1960, 1963), birds (Boag, 1987; Cooch et al., 1991; Johnson, 1971), reptiles (Madsen and Shine, 2000), fish (Reznik, 1990) and arthropods (Atkinson, 1995). In insect parasitoids, the size of an individual is determined by host size as it restrains the amount of food available during development. In *Trichogramma euproctidis*, males have a difference of around 32% in their cephalic capsule size depending if they developed in small (*Plutella xylostella*) or large hosts (*Trichoplusia ni*) (Boivin and Lagacé, 1999). In *Trichogramma pretiosum*, there is a difference of around 21% in tibia length if they develop in *Sitotroga cerealella* or *Helicoverpa zea* (Kazmer and Luck, 1995). In *Aphidius ervi*, a difference of up to 50% occurs in their dry weight depending on the instar of the host (Sequeira and Mackauer, 1992). Parasitoids are thus a good model to study phenotypic plasticity related to size by controlling host size.

Size is known to have important impacts on the life history traits of insects, and consequently on fitness. The larger an insect is, the higher its longevity (Butlin and Day, 1985; Hardy et al., 1992) and fecundity (reviewed by Honek, 1993). Reproductive and resource exploitation abilities can also be affected by size in both

* Corresponding author. Present address: FR2116 CAREN/UMR CNRS 6553 ECOBIO, Université de Rennes I, Campus Beaulieu, 35042 Rennes, France. Tel.: +33 06 43 73 24 91.
E-mail address: veronique.martel@mail.mcgill.ca (V. Martel).

0022-1910/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.01.018

males and females. It has been shown in insects that the number of eggs produced by a female (reviewed by Honek, 1993; O'Neil and Skinner, 1990) and the size of eggs (reviewed by Bernardo, 1996; O'Neil and Skinner, 1990) can be affected by the size of the female. Female *Lariophagus distinguendus* have a four-fold difference in oviposition rate depending on their size. Larger females spend less time and energy ovipositing in *Pimpla nipponica* (Ueno, 1999). It has also been shown that larger males produce more sperm (Bangham et al., 2002; Berrigan and Locke, 1991; Lacoume et al., 2006; Wiernasz et al., 2001). Moreover, larger males can inseminate more females (Boivin and Lagacé, 1999) and win more often when in competition with small males (Lacoume et al., 2006). The phenotypic plasticity in size thus clearly affects the reproductive characteristics of both sexes in insects.

In this study, we investigated the phenotypic plasticity of reproductive traits in an egg parasitoid, *T. euproctidis* Girault (Hymenoptera: Trichogrammatidae). Adults of different sizes were produced on different host species. The reproductive traits considered were the volume of the storage organs (seminal vesicles or spermatheca), the capacity of these storage organs (number of sperm) and the size of the gametes for both males and females. Although reproductive traits like volume of the storage organs and the capacity of these organs are expected to increase with adult size, for traits like gamete size, the predictions are not so obvious. To our knowledge, this is the first study to look at phenotypic plasticity in both male and female reproductive traits, including gamete size, of the same species in a parasitoid.

2. Materials and methods

2.1. General methods

The *T. euproctidis* (Genbank accession number HM116410) used in this study originated from Egypt and has been in culture for more than 20 years. The culture was maintained at 25 ± 2 °C, 50% RH, and L16:D8 on eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Cold-killed *E. kuehniella* eggs were glued on strips made of 3 M Post-it® message pads. All experiments were conducted under the same environmental conditions as the rearing.

2.2. Adult size

Three adult sizes were obtained by using three different host sizes. Small, medium and large *Trichogramma* were obtained by using individuals that developed solitarily respectively from *P. xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Px), *E. kuehniella* Zeller (Lepidoptera: Pyralidae) (Ek) and *T. ni* (Hübner) (Lepidoptera: Noctuidae) (Tn) eggs from our culture. The volume of these eggs is respectively of 0.13 mm^3 (Pak et al., 1986), 0.28 mm^3 (Bai et al., 1992), and 0.62 mm^3 (Harcourt, 1957). To obtain the small and large individuals, mated *T. euproctidis* females, less than 24 h old, were randomly taken from the culture and placed in Petri dishes with more than 20 *P. xylostella* or *T. ni* eggs that were less than 24 h old. After 7 days, the black eggs (the parasitized ones) were isolated in 300 μl Beem® polyethylene capsules to prevent individuals from mating following emergence. Only individuals developing singly from a host egg were used. The medium size *Trichogramma* were obtained by isolating parasitized *E. kuehniella* eggs from the culture in 300 μl Beem® polyethylene capsules.

2.3. Preliminary test: hosts nutritional quality

As both quantity (host size) and quality (host composition) of food contained in hosts could affect development, we quantified the amount of lipids, sugars and proteins of both healthy hosts, and hosts after the emergence of solitary individuals, in order to remove the quality effect from our study. To obtain the quantity of nutrients consumed by the parasitoid during its development, we subtracted the amount of each nutrient in hosts after emergence of a solitary parasitoid from the amount of each nutrient in healthy hosts. To quantify the lipids and sugars present in the host eggs, samples (n = 100 eggs by sample, 3 samples) were crushed in 500 μl of chloroform–methanol (1:2) with a micro-pestle in Eppendorf tubes. After centrifugation at 500 g, 4 °C for 15 min, the lipids and sugars remain dissolved in the supernatant.

To quantify lipids, 40 μl of the supernatant was transferred into an Eppendorf tube and then placed in a water heating block at 90 °C to completely evaporate the solvent. 40 μl of 95% sulphuric acid was added and the tube reheated at 90 °C for 10 min. After 5 min cooling, 960 μl of phosphoric vanillin reagent was then added (van Handel, 1985a). After 10 min, each sample was transferred in a microcuvette and analyzed in a spectrophotometer at 525 nm.

To quantify sugars, 40 μl of the supernatant was transferred into an Eppendorf tube with 960 μl of anthrone reagent and was heated at 90 °C for 10 min (van Handel, 1985b). After 5 min cooling, each sample was transferred in a microcuvette and analyzed in a spectrophotometer at 625 nm.

To transform absorbance values into concentrations, calibration curves were made with standard vegetable oil and glucose (Sigma–Aldrich, St Quentin Fallavier, France) for lipids and sugars analyses respectively.

Analysis of proteins was carried on a separate set of samples because it cannot be made on the same samples as those used for lipids and sugars determinations. Each sample (n = 100 eggs by sample, 3 samples) was crushed in 1 ml phosphate buffer 1 M pH = 7.4 containing 0.001% Triton X-100 (Sigma–Aldrich). Tubes were placed at 4 °C for 5 days to allow time for the Triton X-100 to dissolves the proteins. To quantify proteins, 20 μl of sample was transferred into an Eppendorf tube with 780 μl of water and 200 μl of Bradford Reagent (Bio-Rad Laboratories, Munich, Germany). After 15 min at room temperature, each sample was transferred in a microcuvette and analyzed in a spectrophotometer at 595 nm. Calibration curve was performed with bovine serum albumin (Sigma–Aldrich).

2.4. Dissection

All individuals were dissected less than 24 h after emergence (males: n = 25, 20, 20; females, n = 19, 20, 15 for each size respectively). The right hind leg was first removed from the insect, and the hind tibia length (from the junction with the femur to the base of the tibial spine) was measured with a microscope connected to a computer and recorded using image analysis software (Image Pro®, Media Cybernetics, Maryland), at 640×. The males' seminal vesicles and females' spermatheca were extracted from the abdomen on a microscope slide under a binocular magnifying glass at 128×. The organs were then measured as described above. The seminal vesicles of *T. euproctidis* are composed of two chambers: an anterior globular sac-like dilatation and a posterior pouch called the vesicular pocket (Damiens and Boivin, 2005). To determine the volumes of both chambers, the length and diameter of the anterior chamber and the vesicular pocket of both seminal vesicles were measured. Measurements were taken as described above. The volume (in μm^3) of each chamber was calculated using the equation volume = $4/3(\pi ab^2)$, where a is half the length and b is half the width of each chamber and then the sum of the two chambers was calculated for each male.

The spermatheca of *T. euproctidis* is ampulla-shaped. The volume was estimated as the volume of the large part of the