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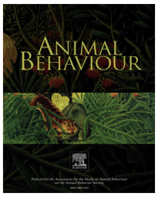

Sex ratio variations with temperature in an egg parasitoid: behavioural adjustment and physiological constraint

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Sex allocation in haplodiploid arthropods is a central fitness-related decision that has received much attention in insect parasitoids. The effect of temperature on the reproductive strategy of female parasitoids has rarely been addressed, despite evidence of its influence on other fitness-related traits. We explored mechanisms inducing the higher production of males typically observed at low and high temperature in parasitic wasps, considering that this pattern may result from both behavioural adjustment and physiological constraint. By observing the oviposition behaviour of an egg parasitoid, *Trichogramma euproctidis*, we were able to distinguish the sex ratio intended by the female from the secondary sex ratio, thereby discriminating between a change in behaviour and a physiological constraint on egg fertilization. More males emerged from eggs laid at low (+45%) or high (+80%) temperature than at medium temperature, but the underlying mechanisms differed between the two conditions. We observed a behavioural change in sex allocation at high temperature, suggesting that laying of sons may be advantageous at high temperature. At low temperature, the females' intended sex ratio was similar to that at medium temperature, but physiological constraints prevented egg fertilization during oviposition, resulting in more males emerging from eggs intended to be females. To our knowledge this is the first experimental evidence that temperature modulates both sex allocation and physiological constraints in egg fertilization in parasitoids.

Sex allocation is a central fitness-related decision that should be selected in response to environmental factors, as predicted by theory and confirmed in many empirical studies (Charnov, 1982; West, 2009). Despite the role of temperature on life history traits (e.g. Angilletta, Steury, & Sears, 2004; Huey & Kingsolver, 1989; Nylin & Gotthard, 1998) and behaviours (e.g. Le Lann, Wardziak, van Baaren, & van Alphen, 2011; Traniello, Fujita, & Bowen, 1984), only a few studies have proposed theoretical models or empirical approaches to understand how organisms should adjust their sex allocation in response to this environmental factor. Among them, Werren and Charnov (1978) developed a perturbation and a cyclical model, assuming that parents can adjust the sex ratio of their progeny. In their perturbation model, they predicted that, if periods of exceptional mortality during cold or heat waves occur and that mortality of one sex is higher than the other, mothers would gain by biasing sex allocation towards the sex with the highest mortality because of the expected reduced competition in the next generation. Alternatively, Roy, Brodeur, and Cloutier (2003) suggested that a change in sex allocation might be adaptive under such dramatic perturbations when one sex has better capacities than the other to disperse and survive under harsh conditions. The cyclical model developed by Werren and Charnov (1978) predicted that adjustment of sex allocation in response to seasonal factors such as temperature should be selected if the relative reproductive success of males and females differs seasonally (Werren & Charnov, 1978). Based on this model, it is also likely that organisms may adjust their sex allocation according to microclimates encountered in their habitat.

Temperature can also physiologically modulate sex ratio. Temperature-dependent sex determination during embryonic development is known in species from the 'reptiles' group (e.g. Ewert, Jackson, & Nelson, 1994; Lang & Andrews, 1994). In some birds (Göth & Booth, 2005) and insects (Wilkes, 1959), developmental temperature is also known to cause differential mortality between males and females, resulting in biased sex ratio. In haplodiploid arthropods, the fertilization of an egg produces a female offspring, while no fertilization results in a male (Flanders, 1956).

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Temperature can thus physiologically influence sex ratio by sterilizing males or incapacitating sperm (King, 1987; Nguyen, Bressac, & Chevrier, 2013; Wilkes, 1959, 1963). Such an effect of temperature on egg fertilization implies that the primary sex ratio (i.e. the sex ratio at oviposition) or the secondary sex ratio (i.e. the sex ratio at emergence of the adult) may differ from the intended sex ratio, the sex ratio 'decided' by the mother, because a physiological constraint occurs between the decision of the female and the end of the oviposition process. If a physiological constraint prevents egg fertilization, a son may be produced from an egg expected to be a daughter by the mother.

Among animal taxa, parasitoid Hymenoptera is one of the most studied groups when investigating sex allocation variations with environment (Ode & Hardy, 2008). This is mainly explained by their reproductive system: mated females store sperm in their spermathecae and are generally able to control the sex of their progeny at oviposition by releasing or retaining sperm (Flanders, 1956). Typically, more males are produced when parents and/or eggs are exposed to low or high temperatures (reviewed in: Colinet & Boivin, 2011; King, 1987), although some species produce a constant sex ratio across a large range of temperatures (e.g. Ichiki, Takasu, & Shima, 2003; Jacas, Peña, Duncan, & Ulmer, 2007). However, the mechanisms underlying sex ratio variations at low and high temperatures have rarely been investigated (King, 1987). By comparing intended sex ratio with primary or secondary sex ratio across a temperature gradient, one may discriminate between an ovipositional decision by the female and a physiological constraint on egg fertilization.

The main challenge when studying sex allocation is to discriminate between sex allocation and progeny sex ratio. Several methods have been proposed over the last decades, including genetic analyses (e.g. Khidr, Mayes, & Hardy, 2013; Ratnieks & Keller, 1998) or the observation of female behaviour during oviposition in species where the oviposition behaviour differs with the sex of the progeny deposited (e.g. Cole, 1981; Suzuki, Tsuji, & Sasakawa, 1984; van Dijken & Waage, 1987). In the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), when a female offspring is deposited, there is a pause in abdominal movements during oviposition, which corresponds to fertilization of the egg by a spermatozoid. When a male progeny is deposited, no such pause is observed and the abdominal movements are continuous (Martel & Boivin, 2007; Suzuki et al., 1984). It is thus possible in these species to calculate an intended sex ratio by observing the oviposition sequence of a female and subsequently comparing it to the primary or secondary sex ratio.

We investigated the effect of temperature during female oviposition on offspring sex ratio in the egg parasitoid *Trichogramma euproctidis* Girault. We compared the intended sex ratio to the secondary sex ratio of progeny laid at three temperatures to explore the behavioural and physiological mechanisms involved.

METHODS

Trichogrammatidae are minute endoparasitoids that usually parasitize eggs of a wide range of Lepidoptera species. The *Trichogramma euproctidis* (Girault) strain used in this study originated from Egypt and was maintained at 24 ± 1 °C, 50% RH, and a 16:8 h light:dark cycle on cold-killed eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Using cold-killed eggs is common in studies on *Trichogramma* and does not affect oviposition behaviour of females nor quality of their progeny (Daumal & Boinel, 1994; Smith, 1996). In *Ephestia kuehniella* eggs, *T. euproctidis* only lays one egg per oviposition sequence. Superparasitism may occur but it requires a second oviposition sequence.

Sex Ratio Experiment

We measured intended and secondary sex ratio of *T. euproctidis* at three temperatures during oviposition: 14, 24 or 34 °C. We considered that a 10° variation above and below the average temperature (24 °C in our colony) is likely to occur during a cold wave or a heat wave, as well as across a day or across microhabitats. Such variation in temperature does not induce any short-term lethal effects on parasitoids, and females remain active despite changes in walking speed (Moiroux, Louâpre, Barrette, Brodeur, & Boivin, n.d.). We collected parasitized eggs from the colony and isolated them in 300 μl Beem® polyethylene capsules with a drop of honey. The next morning, freshly emerged females were mated once with a virgin male, stored at 24 °C for 1–5 h and transferred to a climate room at 14, 24 or 34 °C for 1 h before observation. After this acclimation period, we offered each female 20 cold-killed *E. kuehniella* eggs, placed side by side in four lines of five eggs on a filter paper disk. The experimental arena was delimited by the plastic end piece of a camera (20 mm diameter) covered with Fluon® to prevent females from climbing. Ovipositions were recorded for 30 females per temperature with a camera (Dino-lite Pro AM413ZT, x 60) and analysed using The Observer XT software (Noldus, Wageningen, The Netherlands). Oviposition sequences were divided into three components: host drilling, egg deposition (indicated by abdominal movements) and, if any, pause in abdominal movements during egg deposition (i.e. when an egg was fertilized). During these pauses, the whole body typically stopped moving for a few seconds (see Supplementary Video S1). We determined the sex of each egg deposited, based on the absence or presence of a pause in abdominal movements, and calculated the intended sex ratio. This protocol has been successfully used in several *Trichogramma* species (e.g. Suzuki et al., 1984), including *T. euproctidis* (Martel & Boivin, 2007, named *Trichogramma turkestanica*; Martel, Doyon, & Boivin, 2010). We also recorded duration of pauses in the oviposition sequence to ensure that they were long enough, especially at 34 °C, to be accurately observed. Superparasitism was quantified when a second oviposition sequence was observed on a parasitized host. Observation stopped when a female walked off the patch for more than 3 min. Wasps were thus exposed to 14, 24 or 34 °C for 1 h before observation and during patch exploitation, which increased as temperature decreased. At the end of an observation, we removed the wasp, then isolated the eggs in 300 μl Beem® polyethylene capsules and incubated them at 24 ± 1 °C until emergence to determine the sex of the emerging adult. The sex of each emerging wasp was compared to the intended sex observed during oviposition. The average secondary sex ratio of each ovipositing female was then compared to its intended sex ratio. Superparasitized hosts were not included in the calculation of intended and secondary sex ratios as we were not able to directly compare intended and emerging sex of a given egg. Hatching success was measured for both sexes to examine whether temperature during oviposition had a differential effect on male and female egg survival, which may explain differences in secondary sex ratio.

Survival Experiment

A behavioural change in sex allocation in response to temperature may have been selected for by sex-dependent mortality during a cold wave or a heat wave (Werren & Charnov, 1978). To test this hypothesis, we measured survival to emergence of female and male parasitoids developing at 14, 24 or 32 °C (*T. euproctidis* failed to emerge at 34 °C). Using only mated females would have limited the number of sons produced, since sex ratios were close to 0.13 at 24 °C. We thus used virgin females to produce sons. Sixty mated and virgin females were allowed to parasitize 20 eggs each at 24 °C.