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Defense strategies used by two sympatric vineyard moth pests

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ABSTRACT

Natural enemies including parasitoids are the major biological cause of mortality among phytophagous insects. In response to parasitism, these insects have evolved a set of defenses to protect themselves, including behavioral, morphological, physiological and immunological barriers. According to life history theory, resources are partitioned to various functions including defense, implying trade-offs among defense mechanisms. In this study we characterized the relative investment in behavioral, physical and immunological defense systems in two sympatric species of Tortricidae (*Eupoecilia ambiguella, Lobesia botrana*) which are important grapevine moth pests. We also estimated the parasitism by parasitoids in natural populations of both species, to infer the relative success of the investment strategies used by each moth. We demonstrated that larvae invest differently in defense systems according to the species. Relative to *L. botrana*, *E. ambiguella* larvae invested more into morphological defenses and less into behavioral defenses, and exhibited lower basal levels of immune defense but strongly responded to immune challenge. *L. botrana* larvae in a natural population were more heavily parasitized by various parasitoid species than *E. ambiguella*, suggesting that the efficacy of defense strategies against parasitoids is not equal among species. These results have implications for understanding of regulation in communities, and in the development of biological control strategies for these two grapevine pests.

1. Introduction

Natural enemies including parasitoids are the major biological cause of mortality among phytophagous insects (Hawkins et al., 1997). In response, preys have evolved a set of defenses, including behavioral, morphological, physiological and immunological barriers (Greeney et al., 2012; Gross, 1993). Behavioral mechanisms are the first line of defense, and involve a wide range of behaviors (e.g. biting, twisting, dropping) that act to reduce the risk and effects of infection by parasitoids (Greeney et al., 2012). The second line of defense in insects is the tough cuticle, which forms an efficient protective integument over the external surface (Cole, 1959; Greeney et al., 2012). If the cuticle is breached by parasitic infection, the insect's immune system has to produce a rapid and efficient response to ensure host survival. Insect immunity is innate and relies on a suite of systemic responses that include encapsulation, whereby haemocytes form a multi-layered capsule around a foreign object, such as a parasitoid egg. The agglutinated haemocytes produced melanin on the surface of the capsule, through activation of the enzymes of the prophenoloxidase cascade (Cerenius and Soderhall, 2004; Siva-Jothy et al., 2005).

As described above, the host has several defense strategies for preventing deadly infection. Dewitt and Langerhans (2003(DeWitt, 2003 #235)) proposed an integrated approach to study of the various defense traits, so as to achieve a better understanding of how natural enemies result in the formation of an arsenal of defenses in prey species. Indeed, they noted that different defenses can be either negatively correlated (trait compensation) or positively correlated (trait co-specialization). If defense strategies are costly, it is likely that hosts will evolve only a subset of those available. The cost of defense has some direct support (Flenner et al., 2009; Kraaijeveld et al., 2002; Nelson, 2007; Parker et al., 2011; Rigby and Jokela, 2000). Numerous studies demonstrate trade-offs between morphological and behavioral defenses (DeWitt et al., 2000; Hammill et al., 2010; Mikolajewski and Johansson, 2004; Parker et al., 2011; Steiner and Pfeiffer, 2007), and some a trade-off between behavioral and immunological defenses (Rigby and Jokela, 2000; Zylberberg et al., 2013). Trade-offs suggests that organisms may benefit from balancing investment in immunological and

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non-immunological defenses, because both entail costs but serve a common function. Hence, trait compensation among defense strategies appears to be a common response to predation avoidance. In contrast, few studies have demonstrated co-specialization among defense strategies (DeWitt et al., 2000; Mikolajewski and Johansson, 2004).

Studies assessing the interaction between defense traits have typically focused on two defense barriers. However, integrating behavioral, morphological and immunological defenses in single studies would greatly extend our understanding of compensation and co-specialization among multiple traits (Steiner and Pfeiffer, 2007). Considering immunological and non-immunological defenses in the same study is necessary for assessing the adaptation of hosts to their natural enemies, but only few studies have adopted this approach (Rigby and Jokela, 2000; Zylberberg et al., 2013). To obtain a complete picture of the relationships among defense traits, comparison of closely related species that share the same environment (same ecological niche and trophic resources) is useful in evaluating environmental influences on defense traits (Mikolajewski and Johansson, 2004), and assessing how generalization in insect defenses has evolved (Greeney et al., 2012).

The two sympatric grapevine moths used in this study are *Eupoecilia ambiguella* and *Lobesia botrana* (Lepidoptera, Tortricidae). These two major grapes pests of European viticulture and are ideal candidates (i.e. same ecology) for assessing how defense traits are related. These species often co-occur together in septentrional vineyards having intermediate hygrometry (dry conditions being a lethal factor for *E. ambiguella* eggs). They also share the same parasite range, comprising a few key parasitoids of their eggs and larvae (Thiéry, 2008). In this study we characterized the relative investment in behavioral, physical and immunological defense systems in laboratory strains of these Tortricidae species. We undertook a similar study using wild collected insects of each species, from a single vineyard. In addition, to assess the relative success of the investment strategies of these moth species among behavioral, physical and immunological defense systems, we estimated parasitism success by their parasitoids.

2. Material and methods

This study conformed to French legal requirements, and to accepted international ethical standards, including those relating to conservation and welfare, and to the journal's policy on these matters. All experiments conformed to the Guiding Principles in the Care and Use of Animals, approved by the Council of the American Physiological Society.

2.1. Insect models and experimental designs

E. ambiguella and *L. botrana* (Lepidoptera, Tortricidae), further referred as *E.a.* and *L.b.*, have a wide geographical distributions and mainly damage all grape bunches development stages (Thiéry, 2008). Depending on the region in Europe, *E. ambiguella* completes 2–3 broods each year and *L. botrana* completes 3–4. The first generation of eggs is laid on the flower buds in spring, and the young larvae bore into the flower buds and aggregate them with silk in larval nests called glomerulae. The second generation of larvae emerges between the end of June and the mid-July according to the climate and the third generation occurs between mid-August and the end of September. The larvae are both polyphagous and can develop on most grape cultivars, and on other plant species (Thiéry, 2008; Thiery and Moreau, 2005).

The laboratory strains of *E. ambiguella* and *L. botrana* used in this study were derived from inbred stock maintained at the French National Institute for Agricultural Research (INRA), Bordeaux, France. For each species the larvae were cultured in groups and maintained under standard laboratory conditions (22 ± 1 °C; 70 ± 10% relative humidity; light/dark photoperiod: 16:8). The larvae were fed *ad libitum* with a semi-artificial diet (Vogelweith et al., 2011). The study involved a total of 502 newly hatched larvae (age < 24 h) of *E.a.* and 433 of *L.b.* These were reared individually in centrifuge tubes containing 1.5 mL of semi-artificial diet, which was sufficient for the larva to complete development (Thiery and Moreau, 2005). The lid of each tube was pierced with a needle to enable air circulation. The larvae were cultured until they reached the 5th larval instar stage, when they were used in experiments to assess defense strategies (physical, behavioral or physiological).

Wild larvae of *L.b.* and *E.a.* were collected in May 2012 on Cabernet Franc grapevines at the Château Brillette vineyard (Médoc, Aquitaine, France). We sampled larvae at the end of larval development (5th instar) from the first generation. The 5th instar was checked by measuring the head capsule width, an easy and accurate indicator for the determination of larval stages in natural populations of these species (Delbac et al., 2010). Only silk nests with larvae inside were removed from the bunches. The two grape pest tested in this study are Tortricids (subfamily) which lay separate and spaced eggs among bunches (Thiery and Gabel, 1993). As a result larvae are not gregarious and larvae are single per nest. Collected larvae were maintained in small polyethylene boxes (60 × 40 × 21.4 cm), fed *ad libitum* on bunches collected from the same locality, and maintained at 24 ± 1 °C, 60 ± 10% relative humidity and natural photoperiod conditions until used in experiments. The larvae were then screened using a binocular microscope to estimate the parasitism rate and parasitism success. The parasitism rate was estimated by recording the presence of parasitoid stings (small melanotic patches) on the larval body surface, and was calculated as the number of larvae having parasitoid stings divided by the number of larvae screened. The parasitism rate enabled us to assess the efficacy of the behavioral and morphological defense strategies. Parasitism success was estimated by keeping larvae individually with their silk nest and cotton soaked in water in small plastic jars (30 mm × 30 mm diameter) pierced with a needle to enable air circulation. Each larva was checked daily until pupation occurred, at which time the chrysalis was carefully removed from the flower bud and placed in a glass tube (70 × 9 mm diameter) stoppered with a cotton plug, and stored under standard laboratory conditions, as described above. The chrysalids were checked daily for adult emergence. Parasitism success was calculated as: number of parasitoids/(number of adult *L. botrana* + number of parasitoids emerged). In this measure of parasitism, we considered all larval endoparasitoids emerging in order to get an overall index of the local selective pressure imposed by parasitoid community.

Field larvae with no parasitoid stings on the cuticle were used to characterize levels of investment in behavioral, physical and immunological defenses, as described above for the laboratory strains.

2.2. Behavioral defenses

We focused on three defense behaviors used by moth larvae to escape predators or parasitoids. We first considered the ability of larvae to move away ('flee') by measuring their movement speed. To this end, each larva was placed in a horizontal gridded plastic sheet (84 × 116 cm) and acclimated for 15 s under the cap of a 50 ml Falcon tube. Following removal of the cap, the number of lines crossed by the larvae was recorded for 90 s, which was the minimum time required for a larva to exit the gridded sheet, estimated in preliminary experiments.

The second defense behavior was the ability of the larvae to repeatedly and rapidly twist ('twisting' defense) in response to a stimulation mimicking a parasitoid sting (Greeney et al., 2012).