Self-medication in insects: current evidence and future perspectives

Jessica Abbott

Department of Biology

Section for Evolutionary Ecology

Lund University

Sölvegatan 37

22362 Lund, Sweden

Phone: +4646 222 9304

Fax: +4646 222 4716

Email: Jessica.Abbott@biol.lu.se

Keywords: pharmacophagy, zoopharmacognosy, social immunity, *Grammia*, *Spodoptera*, *Drosophila*, *Danaus*, *Apis*

Running title: Self-medication in insects

Word number: 6800

Abstract

1. Self-medication is ability to consume or otherwise contact biologically active organic compounds specifically for the purpose of helping to clear a (parasitic) infection or reduce its symptoms. Consumption of these compounds make either take place before the infection is contracted (prophylactic consumption) or after the infection is contracted (therapeutic consumption).

2. An important insight is that self-medication is a form of adaptive plasticity, and as such, consumption of the medicinal substance when uninfected must impose a fitness cost (otherwise the substance would be universally consumed). This distinguishes self-medication from several closely related phenomena such as microbiome effects or compensatory diet choice.

3. A number of recent studies have convincingly demonstrated self-medication within several different, distantly-related, insect taxa. Here I review evidence of self-medication in the wooly bear caterpillar *Grammia incorrupta*, the armyworm *Spodoptera*, the fruit fly *Drosophila melanogaster*, the monarch butterfly *Danaus plexippus*, and the honey bee *Apis mellifera*.

4. These studies show not only that self-medication is possible, but that the target of the medication behavior may in some cases be kin rather than self. They also reveal very few general patterns. I therefore end by discussing future prospects within the field of insect self-medication.

Introduction

Plant-derived organic compounds used in traditional human medicine and in animal self-medication have been touted as a source of information for the discovery of new drugs (Huffman 2003). Although evidence for self-medication in nonhuman animals was initially mostly anecdotal, increased research in this area over the past two decades has resulted in convincing evidence of self-medication in a number of species, such as chimpanzees and sheep (Hutchings *et al.* 2003). It has been known for a long time that some types of insect utilize ingested plant compounds for defense (Ode 2006), but despite this, most researchers writing about animal self-medication previously assumed that this behavior would require learning, and therefore only be present in higher vertebrates (Clayton and Wolfe 1993; e.g. Lozano 1998). Yet in the past five years, several papers have been published demonstrating that self-medication is not only possible in insects, but taxonomically widespread. Some of these studies have been highlighted in a recent short perspectives paper in *Science* (de Roode *et al.* 2013b), but I would argue that the time is also ripe for a more comprehensive summary of the field. Here I will therefore review the current literature on self-medication and discuss possibilities for future research in this area.

An insect's first lines of defense against infection are structural (e.g. cuticle and peritrophic matrix, midgut cell sloughing) and behavioural (e.g. avoidance) (Lundgren and Jurat-Fuentes 2012). Once the cuticle has been breached then the innate immune system, which includes melanization, encapsulation, and the production of antimicrobial peptides (Merkling and van Rij 2012; Smilanich *et al.* 2009b), comes into play. However if none of these defenses are effective, then self-medication may be an alternative. True self-medication has a rather strict definition (Singer *et al.* 2009), and the criteria for demonstrating it have been refined over time. An early paper by Boppré (1984) defined pharmacophagy as the search for and uptake of secondary plant substances, for a purpose other than primary metabolism. This definition obviously applies to self-medication behavior, but may also

include other unrelated phenomena, such as the use of plant compounds as olfactory signals ("perfumes") to attract mates (Boppré 1984; Wee *et al.* 2007). Self-medication is therefore usually defined as the use of organic compounds specifically for the purpose of helping to clear a (parasitic) infection or reduce its symptoms (Lozano 1998). These organic compounds need not necessarily be plant-derived; many sources are possible including fungi, other animals, microbes, etc. The purpose of self-medication may vary in terms of tolerance and resistance. Resistance is an individual's ability to limit parasite burden (either by lowering infection risk and/or infection load), while tolerance is an individual's ability to limit the damage caused at a given infection load (Råberg *et al.* 2007). Most of the research to date on self-medication in insects has investigated effects on resistance (discussed in more detail below), but there is at least one example of increased tolerance (Karban and English-Loeb 1997). It is worth noting that medicinal compounds need not necessarily be ingested; absorption, topical application, and proximity are other viable methods of self-medication (Boppré 1984; Clayton and Wolfe 1993), although here I will talk about consumption or ingestion for the sake of simplicity. A classic list of three criteria that must be met to establish self-medication comes from Clayton and Wolfe (1993):

1. The substance in question must be deliberately contacted.

2. The substance must be detrimental to one or more parasites.

3. The detrimental effect on parasites must lead to increased host fitness.

The second and third criteria are rather self-evident; a substance that does not reduce parasite fitness and increase host fitness can hardly be considered medicinal. However the first criterion is rather important in that it separates behavior for self-treatment from other phenomena such as the role of enemy-free space in determining niche breadth and tritrophic interactions (reviewed in Ode 2006). The problem with these criteria is that they do not include any information about the effect

of the medicinal substance on the infected individual. A substance that is universally beneficial should be consumed whenever encountered, and it is questionable whether it is possible in this case to make any distinction between self-medication and diet choice. Because of this, Singer et al. (2009) further developed the concept of self-medication within the framework of adaptive plasticity. Adaptive plasticity is the ability of an individual to change the expression of a trait in a predictable way relative to an environmental factor, and is expected when there is a trade-off between alternative phenotypes (Pigliucci 2005; Sinervo and Svensson 1998). Singer et al. (2009) therefore argued that the existence of a trade-off is essential for establishing self-medication, and added a fourth criterion to those suggested by Clayton and Wolfe (1993):

4. The substance must have a detrimental effect on the host in the absence of parasites.

The insight that self-medication is a form of adaptive plasticity is an important one, in that it suggests that self-medication (both in insects and in other groups) is likely to be more common than previously thought. Studies of vertebrates have usually identified potentially medicinal compounds by investigating items that are not a part of the normal diet (Clayton and Wolfe 1993; Huffman 2003; Lozano 1998). However a plastic self-medication response could just as easily be a quantitative one rather than a qualitative one, for example by increasing the consumption of specific substances that do make up part of the normal diet (Singer *et al.* 2009). Because insects consume a wide range of biologically active organic substances (Ode 2006), there is in fact likely to be a rather large potential for self-medication in insects.

Self-medication as distinct from other phenomena

By using the four criteria listed above, we can distinguish true self-medication behavior from other related phenomena such as prophylactic consumption or compensatory diet choice. Self-medication requires the consumption of foreign compounds, so recent examples where insects use autonomously produced antimicrobial compounds for food hygiene (Herzner *et al.* 2013) or kin grooming (Tragust *et al.* 2013), although interesting, do not fall under the definition of self-medication. The most important factor in separating self-medication from other behaviors is whether the substance in question is toxic or otherwise detrimental to the consumer, in accordance with criterion 4 above. Here it is important to highlight the importance of dose-dependence. Many compounds that are innocuous or even beneficial at low doses can become toxic or otherwise harmful at high doses. Determining toxicity or other costs of consumption over a range of doses can also be challenging in practice. We can therefore refine criterion 4 to state that the substance must be detrimental to uninfected individuals *when ingested at the level ingested by infected individuals.* A further useful distinction is whether the substance is consumed before or after infection. Although not explicitly included in the criteria listed above, substances that are ingested to prevent infection (prophylaxis) may differ from those that are used to treat an existing infection (therapeutic medication). Using these two factors, we can set up a matrix of four related categories, all of which may influence resistance or tolerance to parasites, but only two of which can be considered self medication (summarized in Figure 1):

1. Non-toxic substances that are consumed prophylactically

This is a very broad category in that it could potentially include almost any food source that increases overall condition or immune function (Behmer 2009). One recent paper demonstrated for example that alkaloids in nectar can reduce pathogen loads in bumblebees (*Bombus impatiens* Cresson (Hymenoptera: Apidae)), with no apparent ill effect on the bees themselves (Manson *et al.* 2010).

However if we restrict the category to non-nutrient compounds, then one interesting instance of this phenomenon could be microbiome effects. Bumblebees (*Bombus terrestris* Linnaeus (Hymenoptera: Apidae)) that were experimentally exposed to the trypanosomal parasite *Crithidia bombi* (Kinetoplastida: Trypanosomatidae) developed much lower infection loads if they had previously been fed a solution containing feces from their nestmates (Koch and Schmid-Hempel 2011), in comparison to control individuals (fed only sugar water) and those that were experimentally inoculated with Gammaprotea bacteria (a dominant component of the bee gut flora, and presumably commensal). Although the exact mechanism limiting *C. bombi* growth in the feces-fed bees is unknown, the intestinal flora of the feces-fed bees was similar to that found in lab colonies and in the field, in contrast to the bees in the other treatments (Koch and Schmid-Hempel 2011). This suggests that gut microbiota play a role in health, and that inoculation with the appropriate flora can reduce the severity of parasitic infections. Similarly, *Wolbachia* (Rickettsiales: Rickettsiaceae) has been found to induce viral resistance in *Drosophila* Fallén (Diptera: Drosophilidae) and *Aedes* Meigen (Diptera: Culicidae) (reviewed in Merkling and van Rij 2012). *Wolbachia* is normally transmitted vertically and this precludes selective acquisition, but some instances of horizontal transmission of are known (Schuler *et al.* 2013; Werren 1997), and these examples at least demonstrate that it is possible for infection with one type of microbe to provide a protective effect against another. Although interesting, one caveat with these examples is that it is unknown whether insects deliberately contact the nectar alkaloids or microbiome elements in question (criterion 1).

2. Non-toxic substances that are consumed therapeutically

When specific substances are consumed as a response to infection, but no cost of consumption is evident, this is an example of compensatory diet choice. For example, infected individuals of the beetle *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) increased protein consumption relative to uninfected individuals, allowing them to offset costs of infection. However elevated

138 protein intake did not appear to reduce fitness in uninfected individuals (Ponton *et al.* 2011).
139 Consumption of different yeast species also affects encapsulation ability of larval *Drosophila*
140 *melanogaster* Meigen (Diptera: Drosophilidae) infected by the parasitoid wasp *Asobara tabida* Nees
141 (Hymenoptera: Braconidae), but had no effect on survival until eclosion (Anagnostou *et al.* 2010).
142 Another possible example of therapeutic consumption comes from Karban and English-Loeb (1997).
143 They found evidence that consumption of poison hemlock (*Conium maculatum* Linnaeus (Apiales:
144 Apiaceae)) increased survival rates of the caterpillar *Platypreia virginalis* Boiduval (Lepidoptera:
145 Erebidae) when parasitized by the tachinid parasitoid *Thelaira Americana* Brooks (Diptera:
146 Tachinidae). However it is unclear whether this is really an example of therapeutic consumption, or
147 of host manipulation by the parasitoid (Singer *et al.* 2009), since both host and parasitoid benefited
148 from increased consumption of poison hemlock; the caterpillar in terms of survival, and the
149 parasitoid in terms of eclosion weight (Karban and English-Loeb 1997). In general, compensatory diet
150 choice might usefully be considered a fallback description for any behaviour that meets the first 3
151 criteria for self-medication, but where the presence of costs (criterion 4) has not yet been
152 established.

153

154 3. Prophylactic self-medication

155 Prophylactic self-medication differs from flexible diet choice for optimal nutrient intake in that the
156 substances consumed must impose a fitness cost (Singer *et al.* 2009). A complication is that
157 demonstrating such a fitness cost might not always be straightforward, as the toxicity of a
158 substance may vary not only with dose but also according to the nutritional status of an individual.
159 Tannic acid is normally harmful when consumed, but locusts provided with an optimal ratio of
160 protein to carbohydrate did not experience any deleterious effects of consuming tannic acid
161 (reviewed in Behmer 2009). In addition, insects may consume non-nutritive secondary plant
162 metabolites not to prevent infection, but because the food source in question most closely matches

their nutrient intake target (Behmer 2009). Both of these factors could make it difficult to definitively establish that prophylactic self-medication is occurring. One can also wonder how frequent prophylactic self-medication is likely to be, given that an uninfected individual which consumes the active substance will always pay the associated fitness cost, yet the risk of infection will probably rarely be 100%. *A priori* we might then expect that prophylactic self-medication will be most likely to occur when the risk of infection is high and the associated cost of consumption is relatively low.

4. Therapeutic self-medication

Therapeutic self-medication differs from prophylactic self-medication in that consumption occurs after infection. As discussed above, this need not necessarily mean that the substance in question is never consumed by uninfected individuals. In fact, most of the recent examples of self-medication in insects (discussed in more detail in the next section) involve increased consumption of substances that occur as part of the normal diet. Because of this, demonstrating therapeutic self-medication is probably simpler in practice than demonstrating prophylactic self-medication. The inclusion of a harmful substance in the normal diet could have many causes, as touched on briefly above, but increased consumption after infection of a substance toxic to both host and parasite is difficult to explain in terms of anything but self-medication.

What are the targets of medication behavior?

Within prophylactic and therapeutic medication, an additional useful distinction can be made between self-medication versus medication of kin (de Roode *et al.* 2013b). For example, social insects are known to engage in a number of behaviors that reduce the risk of infection at the colony level, a phenomenon known as social immunity (Cremer *et al.* 2007). One phenomenon that has

been suggested to be a form of prophylactic self-medication is collection of resin for incorporation into the nest in ants (Castella *et al.* 2008; Chapuisat *et al.* 2007; Christe *et al.* 2003) and bees (Simone-Finstrom and Spivak 2012). Such behavior is clearly an example of social immunity, but it is questionable whether it really is a form of (self or kin) medication or not, since the resin did not seem to have any detrimental effect in uninfected ant colonies (Chapuisat *et al.* 2007). If this is generally the case, then resin collection is rather an example of prophylactic (Castella *et al.* 2008) or therapeutic (Simone-Finstrom and Spivak 2012) consumption (i.e. categories 1 and 2 above) and not self/kin medication *per se*. Somewhat surprisingly, the clearest cases for kin medication come from *Drosophila* and *Danaus* (see next section), and not the social insects.

Recent evidence for self-medication

The most convincing cases of self-medication to date are in wooly bear caterpillars *Grammia* Rambur (Lepidoptera: Arctiidae), armyworms *Spodoptera* Guenée (Lepidoptera: Noctuidae), *Drosophila* fruit flies, and monarch butterflies *Danaus plexippus* Kluk (Lepidoptera: Nymphalidae). In these species, all four criteria for demonstrating self-medication have been met. Some recent publications have also suggested that self-medication may exist in honey bees *Apis mellifera* Linnaeus (Hymenoptera: Apidae), although this is somewhat more ambiguous, since not all four criteria have yet been met. A summary of these 5 cases can be found in Table 1.

It is perhaps unsurprising that self-medication occurs in caterpillars such as *Grammia, Spodoptera* and *Danaus*, given the large literature on the use of toxic host plant secondary metabolites for defense in many species of moths and butterflies (Hunter 2003; Ode 2006). At first glance one might therefore not expect self-medication to occur in *Drosophila*, since fruit flies are repelled by some plant toxins (Mitri *et al.* 2009) and generally feed on non-noxious species (the cactophilic and

mycophagous Drosophilids being an exception; Fogleman and Danielson 2001; Jaenike 1985). However because *Drosophila* live on rotting plant tissue, alcohol is a common component of the natural diet (Gibson *et al.* 1981).

Grammia

Work by Singer and colleagues has revealed evidence for all 4 self-medication criteria above in *Grammia*. *Grammia incorrupta* Edwards (Lepidoptera: Arctiidae) is a generalist species that feeds on a wide range of plants producing pyrrolizidine alkaloids (PAs). PAs are biologically active compounds that are noxious to non-adapted species (Hartmann 1999). When infected with parasitoids, either naturally in or a lab setting, *G. incorrupta* preference for PAs increases (Singer *et al.* 2009; Smilanich *et al.* 2011), consistent with deliberate contact (criterion 1) for the purpose of therapeutic self-medication. Increased PA consumption lowers survival of the tachinid parasitoid *Exorista mella* Walker (Diptera: Tachinidae) in experimentally infected individuals (Singer *et al.* 2009), consistent with detrimental effects on parasites (criterion 2). Parasitized caterpillars that were given the opportunity to ingest PAs had higher survival than control caterpillars (Singer *et al.* 2004; Singer *et al.* 2009), consistent with increased host fitness (criterion 3). Finally, increased consumption of PAs has detrimental effects on growth and survival in unparasitized caterpillars (Singer *et al.* 2004; Singer *et al.* 2009), consistent with a fitness cost of consuming the medicinal substance (criterion 4). Interestingly, infection intensity and infection stage appear to influence self-medication behavior. In the early stages of infection, there was no difference in PA consumption between parasitized and unparasitized caterpillars (Smilanich *et al.* 2011). Similarly, surviving caterpillars infected with only one parasitoid egg did not increase PA consumption, while those infected with two or three eggs did, although the difference was not significant in those infected with three eggs (Singer *et al.* 2009). These results are consistent with innate immunity as the first line of defense, and that self-medication behavior is activated only when the innate immune response has shown itself insufficient to clear the infection (Singer *et al.* 2009). Additional indirect evidence for the importance of the

innate immune response comes from the fact that parasitized caterpillars also show increased consumption of nutritive plants that do not contain PAs (Smilanich *et al.* 2011), particularly in surviving individuals infected with only one egg (Singer *et al.* 2009). This is consistent with compensatory diet choice for increased innate immunity, and shows that self-medication and compensatory diet choice are not mutually incompatible. Additional research on the related species *Grammia geneura* Strecker (Lepidoptera: Arctiidae) and *Estigmene acrea* Drury (Lepidoptera: Arctiidae) indicates that the proximate mechanism activating self-medication behavior in this system is likely to be endoparasite-induced taste alteration, such that PA-containing food sources become more palatable to infected individuals (Bernays and Singer 2005).

Spodoptera

More evidence for self-medication in caterpillars comes from research on armyworms (*Spodoptera*). Lee *et al.* (2006) found that resistance to nucleopolyhedrovirus (NPV; Baculoviridae) increased with increasing food protein content in the caterpillar *Spodoptera littoralis* Boiduval (Lepidoptera: Noctuidae), and that infected caterpillars preferred to eat food containing more protein. In addition, surviving infected larvae chose higher levels of protein than infected larvae that ended up dying, indicating that diet choice was not only active but also adaptive. This demonstrates that insects can flexibly adjust their diet according to infection status. Mounting an innate immune response is presumably costly, so an increased protein intake might offset this cost. When uninfected caterpillars consumed levels of protein that maximized performance in infected caterpillars they exhibited a slight decline in performance, consistent with costs of increased protein consumption (criterion 4). These results have also been confirmed for *S. littoralis* infected with *Micrococcus luteus* Cohn (formerly *lysodeikticus*; Micrococcales: Micrococcaceae)(Cotter *et al.* 2011). In addition, similar results have been obtained from *Spodoptera exempta* infected with the bacterium *Bacillus subtilis* (Povey *et al.* 2009), who also found evidence that the cost was due to increased phenoloxidase activity (an important immune enzyme). A follow-up experiment using *S. exempta* infected with NPV

tracked dynamic nutrient intake over time and also found a general pattern of increased protein intake after infection (Povey *et al.* in press). Interestingly, this study used full-sib families split between treatments and could therefore test for genetic variation in self-medication behavior. They found that the response was very consistent across families, suggesting that there is little variation in the degree of phenotypic plasticity in self-medication in this species (Povey *et al.* in press).

Drosophila

Two recent papers by Kacsoh, Schlenke, and colleagues have also convincingly demonstrated self-medication in *Drosophila*. They used food media containing either zero (control) or 6% ethanol, and found that larvae of *Drosophila melanogaster* exposed to the parasitoid wasps *Leptopilina boulardi* (Hymenoptera: Figitidae) and *L. heterotoma* Thomson (Hymenoptera: Figitidae) exhibited an active preference for food containing ethanol (Milan *et al.* 2012), consistent with criterion 1 (active contact with the substance). Wasps were shown to prefer to oviposit in larvae raised on the control (alcohol-free) medium, and wasp larvae showed higher mortality when in hosts from the ethanol-containing medium (Milan *et al.* 2012), consistent with criterion 2 (detrimental effect of the substance on the parasite). Parasitized flies that were given the opportunity to consume the ethanol-containing medium had higher survival rates than those on the control medium (Milan *et al.* 2012), consistent with criterion 3 (a beneficial effect of the medicinal substance when parasitized). Finally, unparasitized fly larvae had higher mortality on the ethanol-containing medium than on the control medium (Milan *et al.* 2012), consistent with criterion 4 (a detrimental effect of the medicinal substance when unparasitized). It is important to note that the effects of ethanol are dose-dependent; levels below 4% are beneficial in adult *D. melanogaster*, while levels above 4% are detrimental (Chawla *et al.* 1981). The 6% level used by Milan *et al.* (2012) is intermediate relative to natural levels of ethanol, which may range up to 12-15% (Gibson *et al.* 1981). Interestingly, ethanol

was less effective as a medication against *L. boulardi* (a specialist of *D. melanogaster*) than against *L. heterotoma* (a generalist wasp), consistent with host-parasite coevolution in this system.

Apart from the therapeutic self-medication discussed above, *Drosophila* has also been found to practice prophylactic kin medication. Female flies that were exposed to *L. heterotoma* showed a significant preference to oviposit on alcohol-containing media, while unexposed control females preferred to oviposit on the alcohol-free control medium (Kacsoh *et al.* 2013). Remarkably, the preference was consistent for at least 4 days after exposure, and only female wasps induced the preference; when female flies were exposed to male wasps their oviposition preference was unaffected (Kacsoh *et al.* 2013). Consistent with the results in Milan *et al.* (2012), offspring survival was higher on alcohol-free medium in the absence of the parasitoid, but higher on alcohol-containing medium in the presence of the parasitoid (Kacsoh *et al.* 2013). Additional experiments using various mutant strains of flies confirmed that recognition of *L. heterotoma* was visual rather than olfactory (Kacsoh *et al.* 2013). A number of other parasitoid species that infect fly larvae induced similar oviposition preferences (Kacsoh *et al.* 2013). Admirably, Kacsoh *et al.* (2013) also included a phylogenetic perspective, and found evidence not only that multiple species of *Drosophila* can adjust oviposition preference according to the risk of parasitization, but also that the strength of the preference was correlated with ethanol tolerance.

Danaus

Two complementary studies have found evidence that monarch butterflies (*D. plexippus*) also engage in prophylactic kin medication. Although incapable of curing themselves of infections of the protozoan parasite *Ophryocystis elektroscirrha* McLaughlin & Myers (Neogregarinorida: Olindiidae), females that are themselves infected show a preference for oviposition on more toxic species of

311 milkweed *Asclepias* Linnaeus (Gentianales: Apocynaceae) (Lefèvre *et al.* 2012; Lefèvre *et al.* 2010),
312 consistent with criterion 1. More toxic species (i.e. containing higher concentrations of cardenolides)
313 were also found to be more efficient in inhibiting parasite growth (Lefèvre *et al.* 2012; Lefèvre *et al.*
314 2010), consistent with criterion 2. Infected individuals raised on a more toxic species (*A. curassavica*)
315 had longer lifespans and lower parasite loads than those raised on a less toxic species (*A. incarnata*)
316 (Lefèvre *et al.* 2010), and there was a negative correlation between spore load and lifespan across 5
317 different milkweed species (Lefèvre *et al.* 2012), consistent with criterion 3. Finally, uninfected
318 individuals had longer lifespans on the less toxic species (Lefèvre *et al.* 2010), consistent with
319 criterion 4. This is an interesting example of prophylactic kin medication, since in this case the risk of
320 parasitism in offspring is determined using the female's own internal state, in contrast to in
321 *Drosophila*, where visual perception of parasitoids is the cue. Such a determination is feasible in this
322 case, because females shed spores of *O. elektroscirrha* during oviposition but larvae are not capable
323 of avoiding these spores (Lefèvre *et al.* 2012). This means that a female's own infection status is an
324 accurate guide to the risk of infection in offspring.

325

326 ## *Apis*

327 Simone-Finstrom and Spivak (2012) recently demonstrated that honey bees (*A. mellifera*) increase
328 resin collection after immune challenge with the fungal pathogen *Ascosphaera apis* Olive & Spiltoir
329 (Onygenales: Ascosphaeraceae); a therapeutic response which is consistent with criterion 1 above.
330 This result is of interest because although resin collection has previously been acknowledged as a
331 form of social immunity (Cremer *et al.* 2007), it has been found to be prophylactic rather than
332 therapeutic (Castella *et al.* 2008). Resin has been shown to reduce parasite loads in bees (Simone *et*
333 *al.* 2009), consistent with criterion 2. Although it's not clear whether this collection results in a direct
334 survival benefit, bees raised in resin-containing nests invested less in innate immune function, which
335 is consistent with criterion 3 (Simone *et al.* 2009). Thus the only aspect that is missing here is the

cost of resin collection in uninfected colonies; if such a cost can be demonstrated then this behavior can definitely be considered an example of self-medication. Although it's possible that resin collection itself could be a cost in that it is energetically demanding, Simone *et al.* (Simone *et al.* 2009) argued that because only a small proportion of workers forage for resin, this is unlikely to be a major cost on the colony level. Obviously more investigation into possible trade-offs of resin collection are needed.

General patterns in self-medication, and future directions

Looking at Table 1, it is in fact difficult to see any general patterns among these examples of self-medication. The behavior may be employed at either the adult or larval stage, and the substance may be either prophylactic in nature, therapeutic in nature, or both. It may be to benefit self, to benefit kin, or both. Not all of the medicinal substances are what would traditionally be considered toxic and they vary considerably in type and origin. A wide variety of parasite groups invoke the self-medication response. One of the commonalities is that all examples involve the consumption (or collection) of compounds that are considered a normal part of the diet (or the immediate environment, in the case of resin). This may be a reflection of the evolutionary origins of self-medication behavior in insects. Although individual learning of medicinal substances might not be completely out of the question (Moore *et al.* 2013), it seems more likely that most self-medication will be quantitative in nature rather than qualitative (de Roode *et al.* 2013a; Singer *et al.* 2009). For example, ingestion of the medicinal substance may have initially had a different function (an example of adaptive plasticity, rather than plasticity as an adaptation; Gotthard and Nylin 1995), or may be a result of low receptor specificity resulting in coincidental ingestion (Tallamy *et al.* 1999). Many insect species also have large population sizes, short generation times, and naturally encounter or ingest biologically active substances, all of which should favor the evolution of self-medication. Because of this, it seems probable that there are many more instances of self-medication yet to be discovered.

But of course many questions remain. Just how taxonomically widespread is self-medication in insects? Are there relatively many independent origins, or only a few? Is frequent contact with the medicinal substance really a prerequisite for the evolution of self-medication, or can chance and individual learning play a role? Are all types of pathogen amenable to the evolution of self-medication, or only some? Similarly, are only certain types of substances suitable for use in self-medication? These questions simply cannot be resolved without more data. One important future direction will therefore be to evaluate the potential for self-medication in many more taxa, and determine if there are any general evolutionary or ecological predictors of the behavior. Variation in the level of response is seen both at the species level (Kacsoh *et al.* 2013) and at the individual level (e.g. Milan *et al.* 2012; Singer *et al.* 2009), begging the question of the heritability of self-medication traits. The heritability and genetic basis of self-medication behavior should therefore also be an important issue to address in future, since to my knowledge only one study to date has attempted to measure genetic variation in self-medication behavior (Povey *et al.* in press).

As results from *Grammia* show, multiple types of response are likely to be mutually non-exclusive, and the interaction between different responses (such as innate immunity and self-medication) is another fruitful avenue of future investigation. For example, do populations with higher parasite pressure evolve an increased propensity for self-medication, an increased innate immunity, or both? A comparative study on caterpillars has already determined that species with the highest innate immunity have lower rates of parasitism (Smilanich *et al.* 2009b), and work on social insects suggests that an effective colony-level immunity results in the reduction of investment in individual immunity (Cremer *et al.* 2007; Simone *et al.* 2009). Innate immunity and self-medication may therefore trade-off against one another (Smilanich *et al.* 2009a), but this question will require more in depth evaluation. A further question is the proximate mechanism of self-medication. In most cases the

386 mechanism controlling the activation of the self-medication behavior is unknown, although some
387 sort of feedback between internal health state and taste and/or olfactory perception seems likely
388 (Behmer 2009), similar to that found in Bernays and Singer (2005). Although demonstrating a cost of
389 plasticity *per se* is often fraught with difficulties (Pigliucci 2005), it is known that increased learning
390 ability imposes a fitness cost in *Drosophila* (Mery and Kawecki 2003). Could the same be true of the
391 capacity for self-medication, independent of the direct detrimental effects of the medicinal
392 substance itself?

393

394 In short, the evidence that self-medication can and does occur, at least in some insect species, is
395 clear. The data at hand also provide a tantalizing suggestion that self-medication is a widespread and
396 highly variable phenomenon. There is much still to be discovered within this fascinating field, and I
397 am sure that there will be many exciting new developments within the next decade.

398

399 Acknowledgements

400 Valuable input was provided by Anna Nordén, Klaus Reinhardt, two anonymous reviewers, and the
401 associate editor. Financial support was provided by the Swedish Research Council
402 (Vetenskapsrådet).

403

404 REFERENCES

405

406 Anagnostou C, LeGrand EA, Rohlfs M (2010) Friendly food for fitter flies? - Influence of dietary
407 microbial species on food choice and parasitoid resistance in *Drosophila*. *Oikos* **119**: 533-541

408

409 Behmer ST (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology* **54**: 165-187

410

411 Bernays EA, Singer MS (2005) Taste alteration and endoparasites. *Nature* **436**: 476

412

413 Boppré M (1984) Redefining "pharmacophagy". *Journal of Chemical Ecology* **10**: 1151-1154

414

415 Castella G, Chapuisat M, Christe P (2008) Prophylaxis with resin in wood ants. *Animal Behaviour* **75**:

416 1591-1596

417

418 Chapuisat M, Oppliger A, Magliano P, Christe P (2007) Wood ants use resin to protect themselves

419 against pathogens. *Proceedings of the Royal Society of London B* **274**: 2013-2017

420

421 Chawla SS, Perron JM, Radoucothomas C (1981) Effects of ingested ethanol on adult *Drosophila*

422 *melanogaster* (Diptera: Drosophilidae). *Canadian Entomologist* **113**: 315-323

423

424 Christe P, Oppliger A, Bancalà F, Castella G, Chapuisat M (2003) Evidence for collective medication in

425 ants. *Ecology Letters* **6**: 19-22

426

427 Clayton DH, Wolfe ND (1993) The adaptive significance of self-medication. *Trends in Ecology and*

428 *Evolution* **8**: 60-63

Cotter SC, Simpson SJ, Raubenheimer D, Wilson K (2011) Macronutrient balance mediates trade-offs between immune function and life history traits. *Functional Ecology* **25**: 186-198

Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. *Current Biology* **17**: R693-R702

de Roode JC, Lefèvre T, Hunter MD (2013a) Response to "Self medication: a learning process?". *Science* **340**: 1042

de Roode JC, Lefèvre T, Hunter MD (2013b) Self-medication in animals. *Science* **340**: 150-151

Fogleman JC, Danielson PB (2001) Chemical interactions in the cactus-microorganism-Drosophila model system of the Sonoran desert. *Integrative and Comparative Biology* **41**: 877-889

Gibson JB, May TW, Wilks AV (1981) Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variation: ethanol levels in breeding sites and allozyme frequencies. *Oecologia* **51**: 191-198

Gotthard K, Nylin S (1995) Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**: 3-17

449

450 Hartmann T (1999) Chemical ecology of pyrrolizidine alkaloids. *Planta* **207**: 483-495

451

452 Herzner G, Schlecht A, Dollhofer V, Parzefall C, Harrar K, Kreuzer A, Pilsl L, Ruther J (2013) Larvae of
453 the parasitoid wasp *Ampulex compressa* santize their host, the American cockroach, with a blend of
454 antimicrobials. *Proceedings of the National Academy of Sciences, USA* **110**: 1369-1374

455

456 Huffman MA (2003) Animal self-medication and ethnomedicine: exploration and exploitation of the
457 medicinal properties of plants. *Proceedings of the Nutrition Society* **62**: 371-381

458

459 Hunter MD (2003) Effects of plant quality on the population ecology of parasitoids. *Agricultural and*
460 *Forest Entomology* **5**: 1-8

461

462 Hutchings MR, Athanasiadou S, Kyriazakis I, Gordon IJ (2003) Can animals use foraging behaviour to
463 combat parasites? *Proceedings of the Nutrition Society* **62**: 361-370

464

465 Jaenike J (1985) Parasite pressure and the evolution of amanitin tolerance in *Drosophila*. *Evolution*
466 **39**: 1295-1301

467

468 Kacsoh BZ, Lynch ZR, Mortimer NT, Schlenke TA (2013) Fruit flies medicate offspring after seeing
469 parasites. *Science* **339**: 947-950
470
471 Karban R, English-Loeb G (1997) Tachinid parasitoids affect host plant choice by caterpillars to
472 increase caterpillar survival. *Ecology* **78**: 603-611
473
474 Koch H, Schmid-Hempel P (2011) Socially transmitted gut microbiota protect bumble bees against an
475 intestinal parasite. *Proceedings of the National Academy of Sciences, USA* **108**: 19288-19292
476
477 Lee KP, Cory JS, Wilson K, Raubenheimer D, Simpson SJ (2006) Flexible diet choice offsets protein
478 costs of pathogen resistance in a caterpillar. *Proceedings of the Royal Society of London B* **273**: 823-
479 829
480
481 Lefèvre T, Chiang A, Kelavkar M, Li H, Li J, de Castillejo CLF, Oliver L, Potini Y, Hunter MD, de Roode JC
482 (2012) Behavioural resistance against a protozoan parasite in the monarch butterfly. *Journal of*
483 *Animal Ecology* **81**: 70-79
484
485 Lefèvre T, Oliver L, Hunter MD, de Roode JC (2010) Evidence for trans-generational medication in
486 nature. *Ecology Letters* **13**: 1485-1493
487

488 Lozano GA (1998) Parasitic stress and self-medication in wild animals. *Advances in the Study of*
489 *Behavior* **27**: 291-317
490

491 Lundgren JG, Jurat-Fuentes JL (2012) Physiology and ecology of host defense against microbial
492 invaders. Vega, F. E. and Kaya, H. K. (eds). Insect pathology. 2nd[13], 461-480. Academic Press
493 (Elsevier), Amsterdam.

494 Manson JS, Otterstatter MC, Thomson JD (2010) Consumption of a nectar alkaloid reduces pathogen
495 load in bumble bees. *Oecologia* **162**: 81-89
496

497 Merkling SH, van Rij RP (2012) Beyond RNAi: antiviral defense strategies in *Drosophila* and mosquito.
498 *Journal of Insect Physiology* **59**: 159-170
499

500 Mery F, Kawecki TJ (2003) A fitness cost of learning ability in *Drosophila melanogaster*. *Proceedings*
501 *of the Royal Society of London B* **270**: 2465-2469
502

503 Milan NF, Kacsoh BZ, Schlenke TA (2012) Alcohol consumption as self-medication against blood-
504 borne parasites in the fruit fly. *Current Biology* **22**: 488-493
505

506 Mitri C, Soustelle L, Framery B, Bockaert J, Parmentier M-L, Grau Y (2009) Plant insecticide L-
507 Canavanine repels *Drosophila* via the Insect Orphan GPCR DmX. *PLoS Biology* **7**: e1000147
508

509 Moore BD, Foley WJ, Forbey JS, Degabriel JL (2013) Self-medication: a learning process? *Science* **340**:
510 1041
511

512 Ode PJ (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy
513 interactions. *Annual Review of Entomology* **51**: 163-185
514

515 Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and*
516 *Evolution* **20**: 481-486
517

518 Ponton F, Lalubin F, Fromont C, Wilson K, Behm C, Simpson SJ (2011) Host use altered macronutrient
519 intake to circumvent parasite-induced reduction in fecundity. *International Journal for Parasitology*
520 **41**: 43-50
521

522 Povey S, Cotter SC, Simpson SJ, Pum Lee K, Wilson K (2009) Can the protein costs of bacterial
523 resistance be offset by altered feeding behaviour? *Journal of Animal Ecology* **78**: 437-446
524

525 Povey, S., Cotter, S. C., Simpson, S. J., and Wilson, K. 2013. Dynamics of macronutrient self-
526 medication and illness-induced anorexia in virally infected insects. *Journal of Animal Ecology In press*.

527 Råberg L, Sim D, Read AF (2007) Disentangling genetic variation for resistance and tolerance to
528 infectious diseases in animals. *Science* **318**: 812-814
529

530 Schuler H, Bertheau C, Egan SP, Feder JL, Riegler M, Schlick-Steiner BC, Steiner FM, Johannesen J,
531 Kern P, Tuba K, Lakatos F, Köppler K, Arthofer W, Stauffer C (2013) Evidence for a recent horizontal
532 transmission and spatial spread of *Wolbachia* from endemic *Rhagoletis cerasi* (Diptera: Tephritidae)
533 to invasive *Rhagoletis cingulata* in Europe. *Molecular Ecology* **22**: 4101-4111
534

535 Simone M, Evans JD, Spivak M (2009) Resin collection and social immunity in honey bees. *Evolution*
536 **63**: 3016-3022
537

538 Simone-Finstrom MD, Spivak M (2012) Increased resin collection after parasite challenge: a case of
539 self-medication in honey bees? *PLoS One* **7**: e34601
540

541 Sinervo B, Svensson E (1998) Mechanistic and selective causes of life history trade-offs and plasticity.
542 *Oikos* **83**: 432-442
543

544 Singer MS, Carrière Y, Theuring C, Hartmann T (2004) Disentangling food quality from resistance
545 against parasitoids: diet choice by a generalist caterpillar. *The American Naturalist* **164**: 423-429
546

547 Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: increased ingestion of
548 plant toxins by parasitized caterpillars. *PLoS One* **4**: e4796
549

550 Smilanich AM, Dyer LA, Chambers JQ, Bowers MD (2009a) Immunological cost of chemical defense
551 and the evolution of herbivore diet breadth. *Ecology Letters* **12**: 612-621
552

553 Smilanich AM, Dyer LA, Gentry GL (2009b) The insect immune response and other putative defenses
554 as effective predictors of parasitism. *Ecology* **90**: 1434-1440
555

556 Smilanich AM, Mason PA, Sprung L, Chase TR, Singer MS (2011) Complex effects of parasitoids on
557 pharmacophagy and diet choice of a polyphagous caterpillar. *Oecologia* **165**: 995-1005
558

559 Tallamy DW, Mullin CA, Frazier JL (1999) An alternative route to insect pharmacophagy: the loose
560 receptor hypothesis. *Journal of Chemical Ecology* **25**: 1987-1997
561

562 Tragust S, Mitteregger B, Barone V, Konrad M, Ugelvig LV, Cremer S (2013) Ants disinfect fungus-
563 exposed brood by oral uptake and spread of their poison. *Current Biology* **23**: 76-82
564

565 Wee S-L, Tan K-H, Nishida R (2007) Pharmacophagy of methyl eugenol by males enhances sexual
566 selection of *Bactrocera carambolae*. *Journal of Chemical Ecology* **33**: 1272-1282
567

568 Werren JH (1997) Biology of *Wolbachia*. *Annual Review of Entomology* **42**: 587-609
569
570

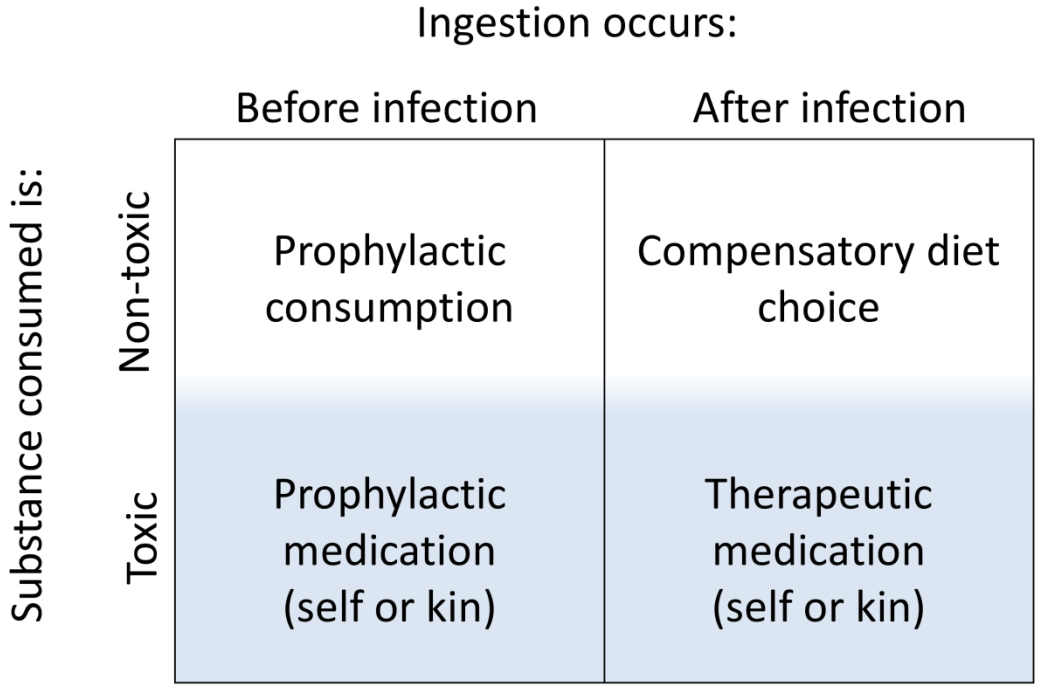
571

572

573 Table 1: Overview of recent studies of self-medication in insects.

Insect	Type of pathogen	Substance used	Costly to consumer?	Prophylactic or therapeutic?	Life stage?	Kin or self?	References
Moth (*G. incorrupta*)	Parasitoid flies	Pyrrolizidine alkaloids	Yes	Therapeutic	Larva	Self	Singer *et al.* 2004, Singer *et al.* 2009, Smilanich *et al.* 2011
Armyworm (*S. littoralis* & *S. exempta*	Virus, bacteria	Protein	Yes	Therapeutic	Larva	Self	Cotter *et al.* 2011, Lee *et al.* 2006, Povey *et al.* 2009, Povey *et al.* in press.
Fruit fly (*D. melanogaster*)	Parasitoid wasps	Ethanol	Yes	Both	Larva	Both	Milan *et al.* 2012, Kacsoh *et al.* 2013
Monarch butterfly (*D. plexippus*)	Protozoan	Cardenolides	Yes	Prophylactic	Larva	Kin	Lefèvre *et al.* 2010, Lefèvre *et al.* 2012
Honey bees (*A. mellifera*)	Fungus	Resin	?	Both	Adult	Kin	Simone *et al.* 2009, Simone-Finstrom & Spivak 2012

Figure 1: Self medication as distinct from other phenomena. Substances consumed may either be prophylactic or therapeutic in nature, but in order to establish true self-medication, four criteria must be met: 1. The substance in question must be deliberately contacted. 2. The substance must be detrimental to one or more parasites. 3. The detrimental effect on parasites must lead to increased host fitness. 4. The substance must have a detrimental effect on the host in the absence of parasites. Assuming that the first three criteria are met, we can develop four categories of related phenomena depending on whether the active substance is contacted before or after infection, and whether it imposes a fitness cost on the consumer or not. Note that although toxicity is highlighted here, this could equally well apply to other forms of fitness cost, and ingestion is not the only method of contacting medicinal substances. Toxicity may also be dose-dependent, which is indicated by the gradual colour gradient between the toxic and non-toxic categories. See main text for more details.



587

588 Figure 1