Asteraceae pollen specialization affects vulnerability to brood parasitism in mason bees (Hymenoptera: Megachilidae)

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Abstract:

Dietary specialization on "toxic" or unfavorable pollens has multiple evolutionary origins in many bee families, despite such pollen being unsuitable for most generalist bee species. Pollen specialization on unsuitable pollen types must confer other evolutionary benefits; an anti-parasitism function may be one. Bees of the family Megachilidae that specialize on composite pollen (of the family Asteraceae) are seldom, if ever, parasitized by sapygid wasps (genus *Sapyga*), a common brood parasite. In this study, we test the effect of composite pollen diets on sapygid larval development and survival. We raised wasp larvae on three pollen types: composite (Asteraceae), legume (Fabaceae) and generalist (a mix of primarily non-composite pollens). Wasp larvae survival was significantly shorter for wasps raised on composite pollen, and larvae failed to reach cocoon-spinning stage. However, for the few composite-reared wasps that reached second-instar, development time to that stage was similar to that of non-composite reared wasps. The decrease in survival suggests that composite pollen specialization is an effective strategy to reduce or eliminate sapygid parasitism.

Introduction:

Reasons for the evolution of pollen specialization (oligolecty) are a long-standing topic of interest in pollination biology. Oligolectic bee species specialize on one or few pollen species, which restricts the resources available to them (Wcislo and Cane 1996; Muller and Kuhlmann 2008; Praz et al. 2008a). However, specialization may confer other benefits. Many species of bee specialize on pollen that is seldom used by other species. For example, several bee species of the family Megachilidae have evolved to specialize on pollen of the plant family Asteraceae, or composite pollen (Williams 2003; Praz et al 2008a). Most generalist (polylectic) bee species are unable to develop when fed a diet of purely composite pollen (Muller and Kuhlmann 2008; Levin and Haydak 1957; Guirguis and Brindley 1974). The fact that most other bees cannot use these pollen sources exclusively (though they may use them as a small component of a mixed diet) may reduce the pressure of competition on specialist species (Wcislo and Cane 1996).

However, these results also suggest that pollen specialists evolve to exploit pollen sources that are toxic or otherwise unsuitable for other bee species. Composite pollens and those used by other specialist bees may be protein poor, lack essential nutrients or necessary enzymes, or contain toxic secondary compounds, though the exact reason for their unsuitability is unknown (Praz et al. 2008a; Muller and Kuhlmann 2008; Roulston and Cane 2000). Even bees that specialize on a given pollen source – i.e. females will collect only that pollen for their offspring – may experience detrimental effects of feeding exclusively on this pollen. Development time for specialist bees can be up to twice as long as that of generalists (Williams 2003; Praz et al. 2008a), and specialist larvae fed their usual specialist diet may reach smaller final sizes than those fed artificial diets containing non-host pollen (Williams 2003). Yet there have been multiple evolutionary origins of specialization on seemingly "toxic" pollen, even within the family Megachilidae (Wcislo and Cane 1996; Muller 1996).

If a particular pollen type is a physiologically challenging protein source even for specialists, the ability to use this pollen must confer other evolutionary benefits. Flowers of the Asteraceae family are abundant and produce large amounts of pollen and nectar rewards (Muller and Kuhlmann 2008), perhaps favoring the evolution of bees that can take advantage of these resources. Here, I propose a novel, complementary hypothesis for the prevalence of composite-pollen specialization: composite pollen may serve an anti-parasite function. Brood parasitism, particularly the type called cleptoparasitism, is common among both solitary and eusocial hymenopteran species (Rosenheim 1989, Munster-Swendsen and Calabuig 2000, Goodell 2003, Cardinal et al. 2010). Cleptoparasites kill the host egg and consume the pollen-and-nectar provision mass collected by the host bee (Goodell 2003, Cardinal et al. 2010). As this form of brood parasitism is almost always fatal to the host egg, it should exert strong selection for counter-adaptations. Parasitism is thought to be one of the driving factors in the evolution of eusociality in some bee species (Rosenheim 1989, Andersson 1984, Lin 1964).

Sapygid wasps, of the genus *Sapyga,* are one of the most common cleptoparasites of bees in the family Megachilidae (Goodell 2003, Munster-Swendsen and Calabuig 2000, Torchio 1972, 1979). Sapygids oviposit into nest cells constructed by megachilid bees. The wasp larva consumes the bee egg upon hatching, then feeds on the pollen mass collected by the host bee (Torchio 1972, 1979). Sapygids can induce up to 77% mortality in some megachilid populations (Torchio 1972), driving strong selection for counter-adaptations. The formation of empty nest cells within the nest is thought to be one adaptation that decreases levels of parasitism by *Sapyga* (Munster-Swendsen and Calabuig 1990). The use of composite pollen may be another counter-adaptation that reduces parasitism rates. Preliminary investigations have demonstrated that composite specialist megachilid bees are seldom, if ever, parasitized by sapygid wasps. In 2008 and 2013, 315 nests from various *Osmia* spp. (Megachilidae) were examined in the area of the Rocky Mountain Biological Laboratory (Colorado, USA). 21.6% of all non-composite nests were parasitized by sapygids, while none of the 19 composite nests showed evidence of parasitism. Sapygid wasps, which are generalists that parasitize several host bee species, may be unable to develop on a pure diet of composite pollen, similar to many bee species.

The aim of this study is to examine whether sapygid wasp larvae are able to develop when fed diets of pure composite pollen, as compared to mixed pollen or pure non-composite pollen. From patterns of sapygid survival and development observed when larval wasps were forced to feed on these pollen types, I infer that the use of composite pollen may confer increased fitness through the prevention of parasitism by sapygid wasps.

Methods:

Study Organism:

Wasp species within the genus *Sapyga* are cleptoparasites of many solitary bee species, predominantly trap-nesting bees of the family Megachilidae. Sapygid wasps oviposit into newly completed nest cells of their hosts. The sapygid larva, upon hatching, consumes the host egg and punctures other wasp eggs that may have been laid in the same nest cell (Torchio 1972). The larva feeds on the pollen provision that was collected by the

host bee, and eventually spins a cocoon in late summer or fall in which it overwinters (Torchio 1972). Sapygid wasps can induce high rates of mortality in host bee populations, and are considered serious pests for important agricultural pollinators such as *Megachile rotundata* (Torchio 1979; Munster-Swendsen and Calabuig 2000).

Study Site and Host Bees:

This study occurred throughout the months of June to August 2014 at sites near the Rocky Mountain Biological Laboratory, approximately 14km north of Crested Butte, Colorado. Wasps were collected from several field sites established in the East River, Coal Creek and Washington Gulch drainages, ranging in elevation from 2700m to 3500m (Figure 1). Nest boxes used by megachilid bees were previously established at all sites by J. Forrest and A. Groulx in 2013 and 2014. Most solitary trap-nesting bees in the region are of the genus *Osmia*, which includes both generalist and specialist bee species. The most common species include: *Osmia iridis,* which specializes on legume pollen (Fabaceae; J. Forrest pers. comm.), a non-composite pollen type that is also used by generalist species; *O. tristella* and *O. tersula*, both pollen generalists; and *O. montana, O. coloradensis* and *O. subaustralis*, all of which specialize on composite pollen. All of these species will readily build nests in wooden nest boxes. Each site was checked twice a week on a rotating basis, and a window was made into the side of all nest cells in order to check for the presence of parasites.

Wasp Development:

Sapygid eggs were collected out of all parasitized nest cells from the established nest boxes. In a few cases, recently hatched first-instar larvae were collected. All eggs or larvae were placed into size 1 gelatin capsules (Capsule Connection, Prescott, AZ) with pollen samples of pure Fabaceae (legume) pollen, generalist pollen (a mixture of primarily non-composite pollens) or pure composite pollen. These pollen samples were collected from *Osmia* nests (unoccupied or abandoned nest cells whenever possible), and the identity of the pollen was determined by microscope examination and comparison with a local reference collection. Due to the small number of abandoned or unoccupied nest cells, pollen samples that had been collected the previous year (2013) were also used. These pollen samples were also collected from *Osmia* nests, and were stored over winter in an incubator kept at 0°C. A very small amount of honey was spread over the base of all gelatin capsules before placing the pollen samples and wasps inside. This was to ensure that the composite pollen, which was dry and crumbling, did not move and crush the wasp larvae when the capsules were disturbed or moved.

The wasps were kept inside the gelatin capsules in an incubator at room temperature (18 - 25°C). All wasps were checked daily throughout the summer. The date of several life stages was noted for each wasp, including the estimated date the egg was laid, date of hatching, date of reaching second instar, date of first defecation, date of the start and the end of cocoon spinning, and, when necessary, date of death (as determined by desiccation or lack of movement when disturbed). These dates were used to determine the number of days from hatching it took each wasp to reach each life stage. As sapygid wasps often take more than one summer to develop into adults, wasp development was considered successful if the wasps reached the cocoon stage, and the number of days to

cocoon stage was considered the final date for survival analyses. If the wasp consumed all of the pollen that had been placed into its gel capsule, more pollen of the same type was provided.

Development Data Analysis:

All data analysis was performed in R. For all analyses, we compared wasps raised on composite versus non-composite pollen, and then further compared wasps raised on composite, generalist, or legume pollen. We also completed all analyses first using all wasps, and then using only wasps that were transferred into gelatin capsules as eggs, to exclude any possible effect of transfer stage. The Kaplan-Meier estimator was used to compare survival among pollen treatments (Kaplan and Meier 1958). We also compared survival between pollen samples collected in 2013 and pollen samples collected in 2014 within the composite and non-composite groups, to ensure that date of pollen collection did not affect survival results. To test for differences in survival among pollen treatments, we used the G-rho family of tests, which are log-rank tests implemented in R using the *survdiff* command (Package survival, T. Therneau 2014). Chi-square tests were used to determine differences in the number of wasps in each pollen treatment that reached second instar. Kruskal-Wallis tests were used to determine differences in the number of days it took wasps in each pollen treatment to reach second instar.

Results:

In total, we raised 26 sapygid wasp larvae: 13 on composite pollen, five on generalist pollen and eight on legume pollen (Table 1). Most pollen samples were collected in 2013 (Table 1). Five wasps were transferred into gelatin capsules as first-instar larvae (Table 1).

Wasps raised on composite pollen were significantly more likely to die than those raised on generalist pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 5.1$, df = 1, p = 0.024, n = 18; Fig. 2) and those raised on legume pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 5.0$, df = 1, p = 0.025, n = 21; Fig. 2). Wasps raised on legume pollen and generalist pollen did not differ in survival (Kaplan-Meier analysis, log-rank test, $\chi^2 = 0.4$, df = 1, p = 0.54, n = 13; Fig. 2). These differences were not as robust when wasps transferred as first-instar larvae were excluded from analyses, though the trends were the same. With first instar transfers excluded, differences in survival were marginally significant between wasps raised on composite and generalist pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 3.3$, df = 1, p = 0.068, n = 15) and between wasps raised on composite and legume pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 3.5$, df = 1, p = 0.060, n = 18). This decrease in significance may be due to a sharp decrease in sample size for generalist and legume pollen. Survival differences between wasps raised on generalist and legume pollen were still not significant (Kaplan-Meier analysis, log-rank test, $\chi^2 = 0.1$ df = 1, p = 0.71, n = 10). A two-way comparison of wasps raised on composite and non-composite pollen (a grouping of both generalist and legume pollen types) demonstrated that wasps raised on composite pollen were significantly more likely to die than those raised on non-composite pollen, regardless

of whether or not first-instar transfers were included (Kaplan-Meier analysis, log-rank test, $\chi^2 > 5$, df = 1, $p < 0.05$; Fig. 2).

The number of wasps that reached the second-instar stage was significantly greater for wasps raised on non-composite pollen compared to wasps raised on composite pollen (Pearson's Chi-squared test, $\chi^2 = 4.82$, df = 1, p = 0.028, n = 26; Table 1). This trend held when wasps transferred as first-instar larvae were excluded from analyses (Pearson's Chi-squared test, $\chi^2 = 5$, df = 1, p = 0.025, n = 21; Table 1). The number of wasps that reached the second instar stage did not significantly differ between wasps raised on composite and generalist pollen (Pearson's Chi-squared test, $\chi^2 = 1.432$, df = 1, p = 0.23, n = 18) or between wasps raised on generalist and legume pollen (Pearson's Chi-squared test, $\chi^2 = 0.023$, df = 1, p = 0.88, n = 13) but was significantly different between wasps raised on composite and legume pollen (Pearson's Chi-squared test, $\chi^2 = 4.3$, df = 1, p = 0.038, n = 21). However, the lack of difference between wasps raised on composite and generalist pollen may be due to the small sample size of wasps raised on generalist pollen (n = 5; Table 1). When wasps transferred as first instar larvae were excluded, the number of wasps that reached second instar differed only marginally between wasps raised on composite and legume pollen (Pearson's Chi-squared test, $\chi^2 = 3.8$, df = 1, p = 0.051, n = 18). The number of days to reach second instar did not differ among pollen treatments, either with or without first-instar larvae transfers (Kruskal-Wallis one-way analysis of variance, $\chi^2 < 0.7$, df = 2, $p > 0.70$; Fig. 3).

Year of pollen collection (2013 or 2014) did not make a difference in any of the analyses conducted. Survival was not significantly different between wasps raised on 2013 composite pollen and 2014 composite pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 0.1$, df = 1, p = 0.81, n = 13) or between wasps raised on 2013 non-composite pollen and 2014 non-composite pollen (Kaplan-Meier analysis, log-rank test, $\chi^2 = 1.1$, df = 1, p = 0.29, n = 13). These trends held when wasps transferred as first-instar larvae were excluded from analyses. Pollen year also did not make a difference in the number of days to reach second instar, both with and without first-instar larvae transfers (Kruskal-Wallis one-way analysis of variance, log-rank test, $\chi^2 < 0.2$, df = 1, $p > 0.7$).

Discussion:

This study demonstrates that sapygid wasps, like many bee species, are unable to develop to maturity when fed a diet of pure composite pollen. Sapygids raised on composite pollen lived considerably shorter lifespans than sapygids raised on legume or generalist pollen. Most composite-fed sapygids were unable even to develop to second instar. However, the composite pollen did not seem to interrupt the course of early larval development. Those composite-fed wasps that did reach second instar were no slower than non-composite fed wasps. This is in contrast to other studies that have found specialist bees tend to develop more slowly than generalists (Praz et al. 2008, Williams 2003). The study otherwise demonstrates clearly that composite pollen is not a suitable food source for sapygid wasp larvae, in addition to showing that generalist and legume pollen sources are roughly equivalent in suitability.

The inability of sapygids to develop on a diet of composite pollen is similar to other hymenopterans (Praz et al. 2008, Muller and Kuhlmann 2008). Most generalist bees collect

little or no Asteraceae pollen (Muller and Kuhlmann 2008) and when those bees are raised on a diet of pure composite pollen, they too are unable to develop to adulthood (Praz et al. 2008). The reasons that composite pollen is unsuitable for most bee species are unknown. Composite pollen may be difficult to digest, or have low protein or nutrient content (Praz et al. 2008, Muller and Kuhlmann 2000, Roulston and Crane 2000). It may lack essential amino acids, or even have toxic properties (Praz et al. 2008, Muller and Kuhlmann 2000, Roulston and Crane 2000). However, despite these drawbacks, specialization on composite pollen has evolved multiple times in multiple families (Muller and Kuhlmann 2000, Wcislo and Cane 1996; Muller 1996).

The inability of sapygid wasps to use composite pollen could provide a strong increase in fitness for specialist bees, something that might drive the evolution of specialization. However, this fitness boost would only occur if composite pollen acted as a deterrent to sapygid oviposition, and did not just prevent full development of the parasite. The wasp must recognize and avoid the nests of composite specialists. Other parasitic wasp species are known to select more resource-rich or advantageous nests when given a choice (Cervo and Turillazi 1996). Given that we have observed no sapygid parasitism in any composite specialist nests, nest avoidance seems likely. Sapygids, like many other wasp species, may use chemical cues to identify appropriate hosts (Lewis and Tumlinson 1988, Turillazzi et al. 2000). More rigorous testing is needed to determine whether composite specialization completely prevents sapygid parasitism.

In addition, the use of small amounts of composite pollen is not necessarily enough to prevent sapygid parasitism. Some generalist nests used in this study contained minimal amounts of composite pollen, and a wasp from one such nest lived past first-defecation stage. Many generalist species are known to collect small amounts of Asteraceae pollen (Muller and Kuhlmann 2008). Interestingly, sapygids do parasitize some species that specialize on other "difficult" pollen types, including *Chelostoma florisomne,* which specializes on pollen of the genus *Ranunculus* (Ranunculaceae) (Munster-Swendsen and Calabuig 2000, Praz et al. 2008). Non-specialist bee species were unable to develop to maturity when fed a diet of *Ranunculus* pollen (Praz et al. 2008). This suggests that sapygids have evolved to use other difficult pollen types, and that the traits that make Asteraceae pollen unsuitable are not necessarily shared among other families with unfavorable pollen.

This study suggests that pollen specialization is another trait, like the construction of empty nest cells, that can reduce levels of brood parasitism. Given that brood parasitism is less common or absent among bees that specialize on composite pollen, and that sapygids are unable to develop on diets of composite pollen, pollen specialization could be an important anti-parasitism strategy. However, further study of sapygid wasp nest choice, and the potential avoidance of composite specialist nests, is needed to give greater credence to this hypothesis. More investigation into specialization, especially the use of difficult pollen sources, could provide great insight into the evolutionary interactions between hymenopteran hosts and their parasites.

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Tables and Figures:

Table 1. Population characteristics of sapygid wasps raised on composite, generalist and legume pollen samples.

Pollen Type	Total Wasps	Pollen Samples from 2014*	First Instar Larvae Transfers	Second Instar**	Days to Second Instar°	First Defecation**	Cocoon Spinning**
Composite	13	4	2	3	5.33 ± 1.53	1	0
Generalist	5	1	2	3	5.50 ± 2.65	1	1
Legume	8	3	1	7	5.33 ± 1.86	3	2

* = All other samples are from 2013

** = Number of wasps to reach this life stage

° = Mean ± Standard Deviation

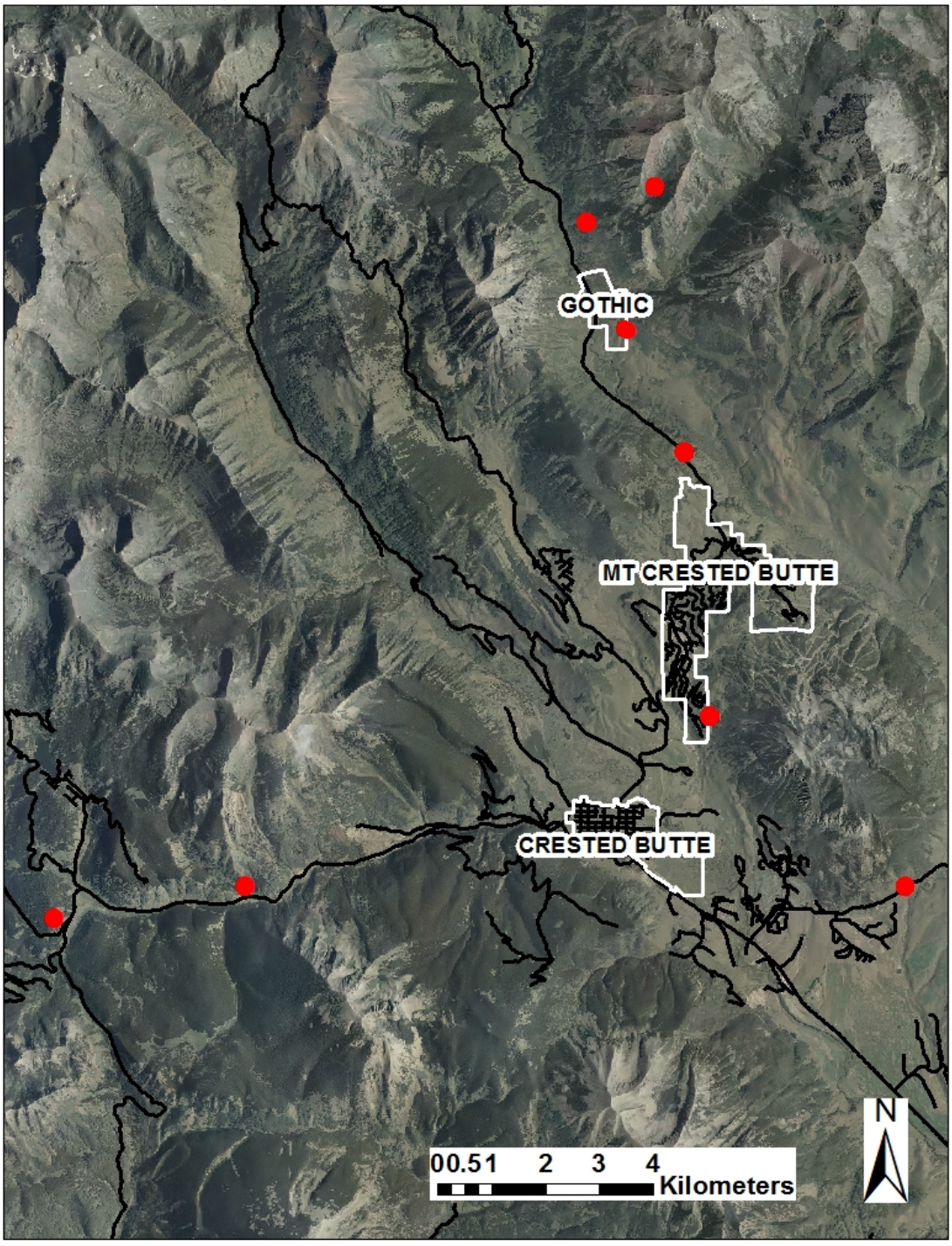

Figure 1. Map of sites near the Rocky Mountain Biological Laboratory where sapygid wasp eggs and larvae were collected. Sites range in elevation from 2700m to 3500m. The towns of Crested Butte, Mt. Crested Butted and Gothic, Colorado are labeled. Roads are outlined in black.

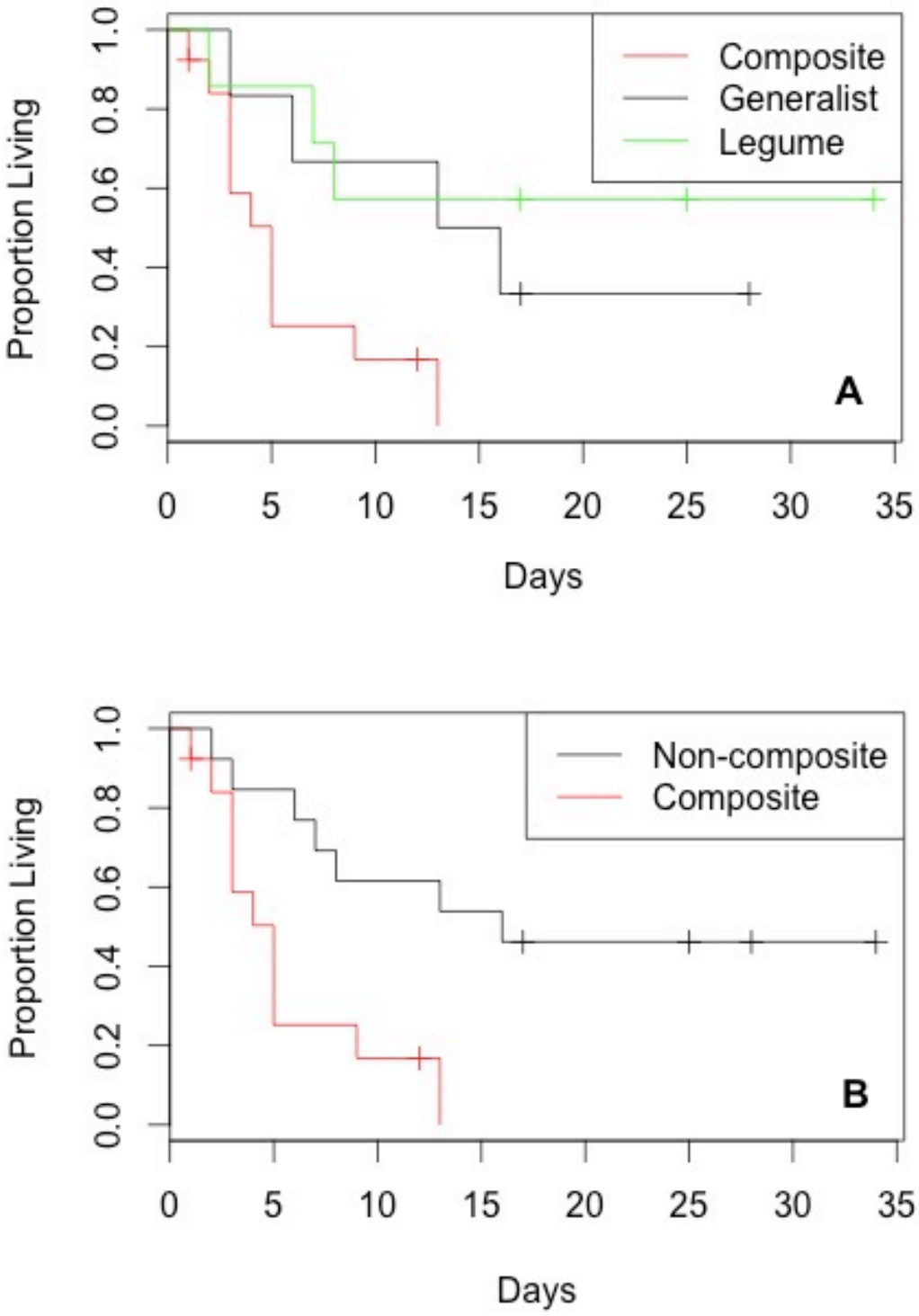

Figure 2. Kaplan-Meier estimator showing differences in survival among wasps raised on (A) composite (n = 13), generalist (n = 5) or legume (n = 8) pollen and (B) composite (n = 13) or non-composite (n = 13) pollen. "Days" represents the number of days wasps survived up to the end of the trial (day 34).

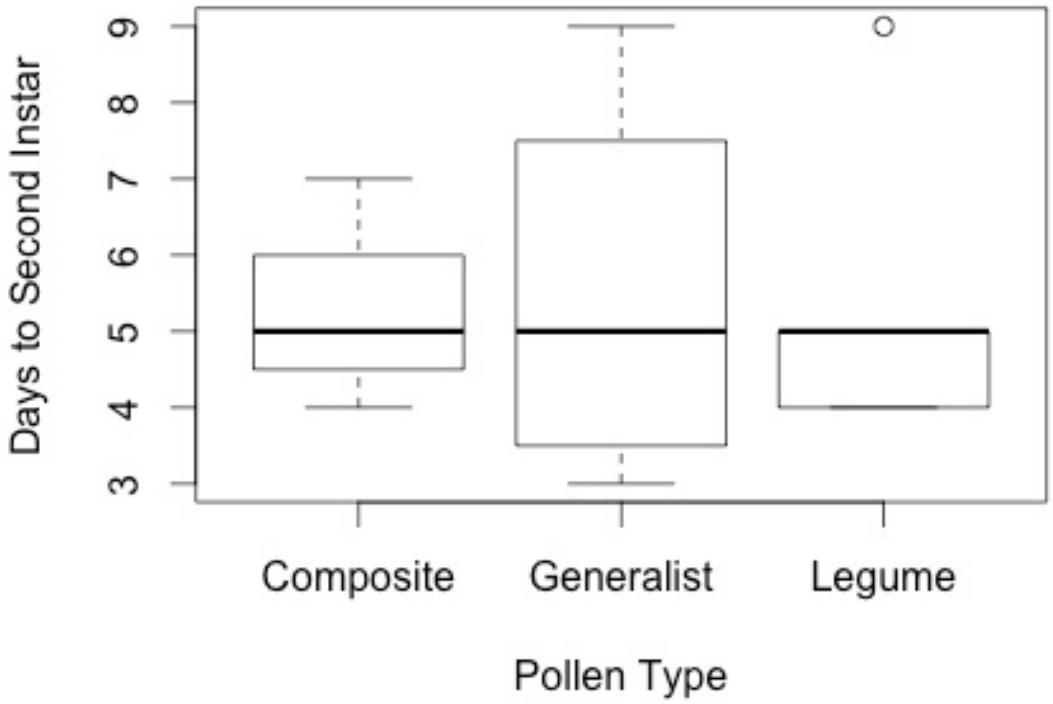

Figure 3. Boxplots of the number of days it took sapygid wasp larvae raised on composite (n = 3), generalist (n = 3) or legume pollen (n = 7) to reach second instar, if the wasp made it to this stage.