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Broski and King: Drilling-in and Chewing-out of *M. domestica* by *S. endius*

Drilling-in and Chewing-out of Hosts by the Parasitoid Wasp *Spalangia endius* (Hymenoptera: Pteromalidae) When Parasitizing *Musca domestica* (Diptera: Muscidae)

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ABSTRACT Many organisms are protected from natural enemies by a tough exterior. Such protection is particularly important for immobile stages, such as pupae. The pupa of some insects is protected by a puparium, which is a shell formed from the exoskeleton of the last larval instar. However, the puparium of certain fly species is drilled through by adult females of the wasp *Spalangia endius* Walker. The female wasp then deposits an egg on the fly pupa within the puparium. After the wasp offspring finishes feeding on the fly pupa, it chews through the puparium to complete emergence. Despite the apparent toughness of the puparium, there was no detectable wear on the ovipositor of *S. endius* females even when females had been encountering fly pupae (*Musca domestica* L.) for weeks, and regardless of whether the pupae were large or old or both. Energy dispersive spectroscopy did not reveal any metal ions in the ovipositor's cuticle to account for this resistance against wear. Offspring of *S. endius* that chewed their way out of pupae also showed no detectable wear on their mandibles. Tests with a penetrometer showed that the force required to penetrate the center of a puparium was greater for larger and for older pupae; and an index of overall thickness was greater for large old pupae than for small old pupae. The lack of an effect of pupal size or age on wear may result from wasps choosing locations on the puparium that are easier to get through.

KEY WORDS host size, host age, resistance, toughness, wear

In host-parasite, plant-herbivore and prey-predator interactions, over time, natural selection will result in populations of organisms evolving defenses to their consumer and populations of consumers evolving countermeasures to those defenses. Physical defenses include mechanical barriers, such as being within a cocoon or gall or having a tough skin or cuticle (Fritz and Simms 1992, Gross 1993, Hanley et al. 2007, Ibanez et al. 2013). However, the effectiveness of this form of defense in deterring consumption or infection depends on the intractability of the external barrier to a particular consumer.

The concept of relative intractability of the external surface as a method of resistance has been especially well-documented in plants, where a hard exterior can successfully deter herbivory, but the concept also applies to host-parasite and prey-predator interactions. Toughness may decrease the chance that a consumer can reach the nutritious parts, decrease digestibility (Massey and Hartley 2009), increase the time required to consume and digest (Clissold et al. 2009, Schofield et al. 2011), and result in more wear on mouth parts, both mandibles and stylets (Raupp 1985, Roitberg et al. 2005, Depieri et al. 2010). In apparent coevolution to such wear, some insect consumers have metal ions, such as zinc or manganese,

incorporated into their outer cuticle (Schofield et al. 2002, Quicke et al. 1998); however, wear is still often evident in older insects (Schofield et al. 2011). In plant-herbivore systems, the degree of wear varies not only among plant species (Depieri et al. 2010), but also with plant age (Raupp 1985). Older leaves tend to result in greater wear on herbivore mandibles. This may result from older leaves having lower moisture (Raupp 1985) and higher concentrations of things like calcium, magnesium (Lin and Wang 2001) and silica (Motomura 2002).

The hardness or toughness of a plant, particularly its outer surface, may affect not only the individuals feeding on it (Clissold et al. 2009), but also the oviposition strategies of their mothers. For example some insects oviposit in or on fruits, on which their offspring then feed; and ovipositing females may chose softer fruits (Follett 2009, Kinjo et al. 2013). A similar situation occurs for parasitoids. By definition, adults are free-living and mothers oviposit in or on other invertebrates, which are killed by the mother at this point or by the offspring's subsequent feeding. That hardness and toughness may affect oviposition success is most obvious for endoparasitoids. However, hosts of ectoparasitoids may also be within a protective covering. Wasps that parasitize fly pupae are ectoparasitoids. The fly pupa is contained within a puparium, which forms from the cuticle of the last larval instar. The adult female parasitoid uses her ovipositor to drill through the puparium to oviposit; but because the egg is laid on the surface of the host pupa, where the subsequent wasp larva remains, the wasps are classified as ectoparasitoids.

The puparium creates problems for a parasitoid in both entry and exit: the mother must drill into the puparium to oviposit, which she does with her ovipositor; and once her offspring finishes feeding, it must exit the puparium, which it does using the teeth on its mandibles (Tormos et al. 2009). The magnitude of these problems may depend on the age and size of the host. Effects of host age may be related to water loss or to chemical changes. As a puparium ages, it loses water; and water loss is associated with increased stiffness and toughness (Vincent and Hillerton 1979, Vincent and Ablett 1987). Just through scaling, the puparium of a large host is likely to be thicker than that of a small host.

Although wear has been demonstrated in herbivores, including insects, relatively little information is available on ovipositor and mandible wear in parasitoid wasps, despite their species richness (Godfray 1994) and the potential effects on their fitness. The present study examined drilling-in and chewing-out problems using the parasitoid wasp *Spalangia endius* Walker (Hymenoptera: Pteromalidae) and one of its hosts, the house fly, *Musca domestica* L. The effects of host age and host size on wear were examined. In *S. endius* only one offspring generally completes development per host (Rueda and Axtell 1985). A female wasp's first drill attempt takes less time on young hosts compared to old hosts (*S. cameroni* Perkins: King 1998) and is more likely to succeed (*S. cameroni*: King 1998; *S. endius*: King 2000). Additionally, once they reach adulthood, a greater proportion of offspring are able to emerge from young hosts compared to old hosts (*S. endius*: King 2000). The drill durations of both *S. cameroni* and *S. endius* are longer in larger hosts (King 1994, 2002). The present study examined whether emergence from a host caused mandibular wear, whether repeated exposure to hosts for three weeks caused ovipositor wear and whether the age and size of hosts affected ovipositor wear. We also looked for metal ions in the ovipositor. Effects of host age and hosts size on some physical properties of *M. domestica* puparia were also examined, specifically, the force required to puncture the puparium and a thickness index.

Materials and Methods

General Methods. The *Spalangia endius* were from a colony originally established with wasps from a poultry farm in Zephyr Hills, Florida. Vouchers are at the Illinois Natural History Survey Center for Biodiversity, Insect Collection 6035 through 6054. Wasps were reared on a natural host, *Musca domestica*, in a 25°C incubator (50-70% RH) with a photoperiod of 12L:12D.

Hosts were reared on a medium consisting of commercial fly larva medium, fishmeal, and pine shavings in 8:1:10 ratios. The commercial fly larva medium consisted of ground oat hulls, ground barley, wheat bran, dehydrated alfalfa meal (Lab Diet, St. Louis, MO; <http://www.labdiet.com>). The density at which hosts were reared was manipulated to produce hosts of different sizes. To produce large hosts,

approximately 800 mm³ of *M. domestica* eggs were placed into a plastic container (31 cm L X 16 cm W X 9 cm H) with 1,030 ml of dry medium mixed with 430 ml of water (King 1988). For small hosts, approximately 350 mm³ of *M. domestica* eggs were put into a round, plastic container (about 7 cm diameter X 5.8 cm height) that contained 100 ml of dry medium mixed with 80 ml of water. Water was added as needed until the larvae pupated. These egg-to-medium ratios produce hosts that differ noticeably in size. Pupae for the young host treatments were parasitized within 24 h of pupariation, and pupae for the old host treatments were set aside for 3 d before being parasitized.

Except where noted, the *S. endius* females used in experiments had developed in large hosts and were 0-1 d old when first given hosts. Within a day of their emergence, the females were isolated from a Petri dish of parasitized hosts from which males had already begun emerging.

For the wear experiments, the head width of each wasp was measured using an ocular micrometer. Wasps were stored in a freezer at -17°C, but were allowed to thaw for at least 5 min before dissection. Ovipositors and mandibles were dissected out using a sharpened #3 insect pin, an angled knife microtool, and forceps under a stereomicroscope.

Ovipositor Wear. To evaluate ovipositor wear, ovipositor dimensions were compared between naïve females (i.e. have not oviposited) and experienced females (i.e. have oviposited). Experienced females were given 10 hosts in a glass vial (2 cm D X 6.8 cm H) each day for 21 d or until death, whichever came first. Each female was given either: (1) small young hosts, (2) small old hosts, (3) large young hosts, or (4) large old hosts. After 21 d, any female wasps that were still alive were killed by being placed in a -17°C freezer until dissection. Naïve females, 0-2 d old, were also killed by freezing to be used as controls. Each female's ovipositor was then removed, photographed, and measured.

To expose the ovipositor for removal from the body, a female was placed on her dorsal surface, and an insect pin was rolled anterior to posterior along the female's abdomen. The ovipositor was then cut from the body at the distal margins of the bulbous articulation (King 1962). The ovipositor consists of an upper valve and two lower valves. The two lower valves have serrations at the distal tip and slide along the upper valve when drilling (Quicke et al. 1994). Care was taken to prevent the lower ovipositor valves from sliding along the upper valve, as this made the serrations difficult to photograph. Cases where the valves separated when dissected out were discarded from analysis. The ovipositors were then transferred to a Scanning Electron Micrograph (SEM) glass slide and coated with gold/palladium using a Polaron SEM Coating System (Quorum Technologies Ltd., East Sussex, UK) and photographed using a JEOL5610LV scanning electron microscope (JEOL Ltd, Herts, UK) at 1000X magnification operating in high vacuum mode. The microscope was operated at 20 kV with a working distance of 12 mm. Two measurements were taken using Image-Pro Plus software, ver. 6.3 (Media Cybernetics, Inc., Silver Spring, MD): the distance between the tips of the second and third serrations and the depth between those serrations (Fig. 1). Each pair of measures was repeated three times. The index of ovipositor wear was calculated by dividing the distance from tip to tip by the depth (Roitberg et al. 2005). A higher index indicates a higher degree of wear. The width of the head was used to control for wasp size. Qualitative analysis for metal ions in the ovipositor was performed using a Thermo-Noran Vantage Energy Dispersive Spectroscopy System (Thermo Noran, Inc., Madison, WI) attached to the scanning electron microscope (n =5).

Mandible Wear. This experiment addressed whether the process of emerging from a host causes wear on the mandibles of wasps. Large old hosts were parasitized by exposure to the wasp colony for 5 d. Large old hosts were chosen because the greater intractability associated with their puparium was expected to result in the highest degree of wear compared to wasps that had not chewed out of the puparium. Parasitized hosts were placed into individual glass vials (1.2 cm D X 7.5 cm H), which were then plugged with cotton.

Once several of the wasps had emerged, half of the remaining isolated pupae were dissected open using a probe under a stereomicroscope, allowing these unemerged wasps to avoid chewing their way through the puparium. The wasps in the remaining pupae were allowed to complete emergence normally. The unemerged wasps served as the control, and the emerged wasps were the experimental group. Only mandibles from female wasps were used. Once they were removed, the mandibles were positioned with their interior surface facing up and the incisors lying flat to show the depth between them. Images were

captured using a Hamamatsu Orca-100 cooled CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan) attached to a macro lens mounted on a Zeiss Axiovert 135 inverted microscope (Carl Zeiss, Jena, Germany). In addition to head width, two measurements were taken from the images using Image-Pro Plus software, ver. 6.3: the distance between the tips of the two incisors of the mandible and the depth of the space between the incisors (Fig. 2). Each measure was taken three times, and the average of the three measures was used for analysis. The index of mandible wear was calculated by dividing the distance from tip to tip by the depth. A higher index indicates a higher degree of wear.

Host Puparia. To evaluate how physical characteristics change with host size, an index of puparium thickness of small and large hosts was estimated (following Fellowes et al. 1998, Morris and Fellowes 2002, Kraaijeveld and Godfray 2003); but using weight of the puparium at the time that it would naturally be oviposited in, not weight after an adult fly had developed and emerged). The thickness index was calculated as the dry mass of the puparium divided by the surface area; thus it provides a general sense of the puparium as a whole and will vary with both thickness and density. Dehydration of the puparium occurs naturally as the host sclerotizes with age; this dehydration results in a decrease in puparium thickness as density increases (Vincent and Hillerton 1979). The effect of host age on the thickness index was not assessed because the index includes puparium weight which necessitates drying the puparium, which might have negated any host age difference. As a result, only the effects of host size could be investigated using this method. Host size effects were examined using old hosts, which were chosen because older pupae can be separated from the puparium with greater ease, ensuring that only the mass of the puparium was measured, without residual pupal tissue. First, pupal length and width were determined using an ocular micrometer on a stereomicroscope. The surface area was calculated using Knud Thomsen's formula for approximating the surface area of a prolate spheroid (Michon 2013), $S = 4\pi [(a^p b^p + a^p c^p + b^p c^p)/3]^{1/p}$ where a is half the length, b is half the width, c is half the height, and $p = 1.6075$. Second, the pupa was dissected out of the puparium, and then the puparium was rinsed with deionized water. Deionized water was used to ensure no minerals were present in the water to be incorporated into the cuticle. The puparium was air-dried overnight and then weighed. The samples were not oven dried because air drying proved to be sufficient; when placed on the microbalance, sample weights remained constant, indicating water evaporation had ceased. The index of thickness of each puparium was estimated by dividing the dry weight by the surface area. This index of thickness is similar to density, but is per surface area instead of per volume.

A penetrometer was built in order to estimate the force required to penetrate the puparium (Fig. 3). A stage (2.5 cm H X 10.3 cm L X 6.4 cm W) was built from balsa wood containing a brass bushing in the center of the stage. A pupa was placed on a block of wood (1.3 cm H X 2.0 cm H X 1.5 cm W) in a divot 0.3 cm deep, which was centered beneath the bushing. A model rocket nose cone (10 cm H X 2.5 cm diameter at top) with a dulled, #3 insect pin (cross-sectional area of 2.83×10^{-3} mm) attached to the tip was threaded through the bushing with the pin resting on top of the pupa. The cone was supported by a PVC pipe. The entire apparatus was placed on top of a microbalance. Sand was slowly added to the cone until the pin punctured the puparium. The weight of the sand plus the cone was used to calculate the force needed to puncture, using the calculation $F = ma$. Twenty pupae from each of the four treatments were analyzed.

Statistical Analyses. Statistical analyses were performed using IBM SPSS Statistics v 21 software (IBM 2012). Alpha was set at 0.05, except where noted. Analyses of variance are robust to assumptions of normality and homogeneity, particularly when sample sizes are very similar, which they were (Zar 2010; Kikvidze and Moya-Laraño 2008). Nevertheless, assumptions of normality and homogeneity were met at alpha of 0.001, with normality examined by the Fisher-Pearson coefficients of skewness and kurtosis or by graphical assessment of normal probability plots of standardized residuals and with homogeneity assessed by Levene's tests.

Repeatability of the ovipositor wear index and mandible wear index were both greater than 90%. The mean of the three measures on each individual was used in subsequent analyses. The effects of host size and age on ovipositor wear index were analyzed using two-way Analysis of Covariance (ANCOVA), with wear index as the dependent variable, host size and age as the independent variables, and head width as the covariate. Because there were no significant effects of covariate or interactions with the covariate,

head width was excluded from subsequent analyses. Specifically, to test the effects of host size and age, a two-way Analysis of Variance (ANOVA) was performed with wear index as the dependent variable and with host size and age as the independent variables. There were no significant effects or interaction effect, so the four experienced treatments appear not to differ and so were combined to increase statistical power and to simplify presentation prior to being compared to the control of naïve females, using one-way ANOVA with wear index as the dependent variable and experience as the independent variable.

Indices of mandible wear were analyzed using repeated measures ANOVA with wear index as the dependent variable, treatment (unemerged or emerged) as the between subject effect and left versus right as a within subject effect. ANOVA is presented in place of ANCOVA because head width of emerged and unemerged females did not differ significantly ($t_{18} < 0.001$, $P = 1.00$; mean \pm se, minimum-maximum: 0.51 ± 0.003 , 0.48 to 0.53). Conclusions were the same when head width was included as a covariate in an ANCOVA.

The effects of host size and age on the force required to penetrate the puparium were analyzed using two-way ANOVA with host size and age as the independent variables. The difference in the indices of thickness of small and large hosts was analyzed with one-way ANOVA, and linear regression was used to evaluate the relationship between the force required to penetrate the puparium and the thickness index.

Results

Ovipositor Wear. Two-way ANCOVA of ovipositor wear among the experienced treatment groups revealed no significant effect due to host age ($F_{1,50} < 0.001$, $P = 0.98$), host size ($F_{1,50} = 0.93$, $P = 0.34$), or head width ($F_{1,50} < 0.001$, $P = 0.99$) (Fig. 4). Additionally, there was no significant interaction effect between host age and size ($F_{1,50} = 0.033$, $P = 0.86$) or host age, host size, and head width ($F_{3,50} = 0.39$, $P = 0.76$). A follow-up two-way ANOVA ignoring head width showed no significant effects due to host age ($F_{1,54} = 0.003$, $P = 0.96$), host size ($F_{1,54} = 0.023$, $P = 0.88$), or interaction between host age and host size ($F_{1,54} = 0.89$, $P = 0.36$). The pooled indices of wear from the four experienced treatment groups were then compared to the control. Ovipositor wear did not differ significantly between naïve and experienced females (Fig. 4; $t_{67} = 1.72$, $P = 0.089$). Energy dispersive spectroscopy did not reveal the presence of any associated metal ions in the ovipositor cuticle ($n = 5$).

Mandible Wear. The wear on mandibles (Fig. 5) was not significantly affected by whether the wasp had been allowed to emerge or not ($F_{1,18} = 2.54$, $P = 0.13$) or by whether it was the left or right mandible ($F_{1,18} = 0.12$, $P = 0.74$), and there was no significant interaction effect between treatment (unemerged or emerged) and left versus right ($F_{1,18} = 2.91$, $P = 0.11$).

Host Puparia. Host volume differed with host size ($F_{1,76} = 1691.52$, $P < 0.001$), but not with host age ($F_{1,76} = 0.01$, $P = 0.92$), and there was no significant interaction effect ($F_{1,76} = 0.008$, $P = 0.93$). Small hosts were $13.72 \text{ mm}^3 \pm 0.30$ (10.59-19.30), $n = 40$; and large hosts were 32.91 ± 0.35 (28.74 – 38.07), $n = 40$. The range of host sizes was comparable to those of parasitized hosts in a natural environment (King 1990).

The force required to penetrate the puparium was significantly affected by both host size and host age (Fig. 6; $F_{1,76} = 246.00$, $P < 0.001$; $F_{1,76} = 15.13$, $P < 0.001$), with more force required for large hosts and for older hosts. There was no significant interaction between the effects of host size and host age ($F_{1,76} = 2.42$, $P = 0.12$).

Among old hosts of the small and large host size categories combined, force to penetrate increased with increasing thickness of the puparium, but thickness explained only 20% of the variance in force (Fig. 7; $R^2 = 0.20$, $y = 6.10x + 0.16$; $F_{1,38} = 9.35$, $P = 0.004$). Looking at small and large hosts separately, force to penetrate was not significantly related to thickness of the puparium (small hosts: $R^2 = 0.17$, $F_{1,18} = 3.71$, $P = 0.07$; large hosts: $R^2 = 0.15$, $F_{1,18} = 3.22$, $P = 0.09$). The mean index of thickness was significantly greater for large hosts than for small hosts (unequal variance t -test: $t_{29,15} = 3.78$, $P = 0.001$). In the large category, hosts with larger volumes had heavier puparia ($F_{1,18} = 5.00$, $P = 0.038$); but in the small category, hosts with larger volumes did not necessarily have heavier puparia ($F_{1,18} = 0.50$, $P = 0.49$). Visual observations suggested that hosts in the small category were more variable in thickness,

with some having translucent puparia. Consistent with this observation, the variance of the index of thickness was greater for small hosts (Fig. 6; $F_{1, 38} = 6.01$, $P = 0.019$).

Discussion

Ovipositing for weeks resulted in no detectable ovipositor wear regardless of the age or size of hosts that females had encountered. Resistance to wear does not appear to result from metal ions in the ovipositor. There also was no evidence that chewing their way out of hosts caused wear on the mandibles of offspring. Physical properties of hosts differed with the host's size and age. An index of overall thickness was greater for large old hosts than for small old hosts; and the force required to penetrate the center of a puparium with a penetrometer was greater for larger hosts and for older hosts.

The lack of ovipositor wear from drilling hosts, even old hosts, helps explain why whether an *S. endius* female has been drilling 0-day-old versus 3-day-old hosts for a week has no significant effect on her offspring production on subsequent days (King 2000). That there was no ovipositor wear in the present study of *S. endius*, which used laboratory-reared females, is consistent with qualitative observations of other hymenopteran specimens collected from the field (Quicke et al. 1998). In contrast, wear is frequently seen in the ovipositor of field collected females of several tephritid species that oviposit in fruits (Jones and Kim 1994). Some tephritids use punctures of other females, and are more likely to do so when the fruits are unripe, suggesting that it may be a mechanism for reducing wear (Papaj and Messing 1996). Because ovipositing females in the present study were not with other females they could not have been minimizing wear by using punctures of other females. However, females sometimes use punctures of other females in the egg parasitoid *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) (Takasu and Hirose 1991).

Ovipositor wear was not detectable in the present study despite the large number of hosts that females encountered. Laboratory females likely have more time to parasitize hosts compared to females in nature, because females in the laboratory do not need to travel far or to burrow through substrate in search of hosts. An *S. endius* female in the laboratory spends more than 22.26 hours drilling within just the first two weeks of emergence, based on the average total offspring production during that time, 80.95, and the mean duration of her first successful drill, 16.50 min (Broski 2013). This estimate is conservative as it only includes the time spent on successful drills, i.e. drills where the ovipositor is fully inserted into the host. Taking into account the average portion of drill attempts that are successful, 0.64, a female is estimated to spend 36.51 hours ovipositing by 14 d of age.

The absence of ovipositor wear in *S. endius* does not appear to result from resistance provided by metal ions. Metal ions were not found in ovipositors of *S. endius* females. Metal ions commonly associated with hardening in insect cuticle (Cribb et al. 2008, Quicke et al. 2004, Schofield et al. 2002), such as zinc or manganese, are incorporated into the cuticle of ovipositors in many hymenopterans (Quicke et al. 1998). The presence of these metal ions may result in an increased resistance to wear (Schofield et al. 2002). *Spalangia* is in Chalcidoidea, a hymenopteran superfamily of mostly parasitoids. Only three out of the eleven chalcidoid species that have been examined have zinc ions incorporated in the ovipositor cuticle, and manganese was not found in any of these eleven species (Quicke et al. 1998). Among hymenopterans as a whole, metal ion incorporation seems to depend on the parasitoids' hosts and their location; zinc and manganese are often present in wasps whose hosts have a tough exoskeleton or are contained within a tough substrate such as wood (Quicke et al. 1998).

In addition to the absence of ovipositor wear, chewing through a host's puparium to emerge likewise did not cause detectable wear on the mandibles of *S. endius*. Mandible wear may not be a problem for *S. endius* because an individual only chews out of a host once, at emergence, and *S. endius* are not known to feed on solids as adults. Many previously documented cases of mouthpart wear are in insects that repeatedly consume a food source with high intractability, e.g., mandibles of a coleopteran feeding on willow leaves (Raupp 1985), stylets of a hemipteran feeding on leaves, stems and insect prey (Roitberg et al. 2005), mandibles of a caterpillar feeding on grasses containing silica (Massey and Hartley

2009), and stylets of a hemipteran feeding on legume pods and seeds (Depieri et al. 2010). Among parasitoid wasps, those that egress from a “hard host substrate, typically in wood,” frequently show wear of their mandibles (Quicke et al. 1998). Metal ions have been found in the mandibles of such wasps, including in some chalcidoids.

A puparium is not of uniform thickness, and *S. endius* females attempt to drill at different locations on a host before finally succeeding (King 1998, 2000, 2001). Whether choice of specific locations on a puparium by females or their offspring prevents wear remains to be tested. When a host has not been parasitized and the adult fly pushes its way out, the puparium splits along specific locations within the anterior half of the puparium (Hewitt 1908). How often this fly-egress location corresponds to where parasitoids drill and egress also remains to be tested.

Controlling for location on the puparium, the penetrometer experiment with *S. endius* showed that puparia of old hosts, and especially large hosts, required the greatest force to penetrate. Regardless of age, large hosts required almost twice as much force to puncture as small hosts, perhaps due to the greater overall average thickness and/or density of their puparia. Differences in penetrability among different locations on a puparium and how such differences relate to host age and host size remain to be tested.

The greater puparial thickness of larger hosts in the present study contrasts with a previous study of *M. domestica* by Roseland et al. (1985), where no effect of host size was found. This difference between studies is likely due to differences in the range of sizes tested. Most of the hosts used by Roseland et al. (1985) were approximately 2.70 mm wide; whereas in the present study, small hosts were 2.08 mm \pm 0.12 wide, and large hosts were 3.00 mm \pm 0.09 wide (Broski 2013).

The present study focused on physical features of the puparium. Once a female drills through the puparium, additional force will be needed as she uses her ovipositor to pierce the exoskeleton of the host within the puparium. However, that exoskeleton is more delicate than the puparium at the stages examined in the present study (Dwivedi and Agrawal 1995). She pierces the exoskeleton of the host itself in order to inject venom prior to ovipositing on the surface (Rivers 2004).

The present study did not test for an effect of a host’s age on its index of thickness. The thickness of puparia of the apple maggot fly *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) increases with age, as does the force required by the parasitoid wasp *Phygadeuon wiesmanni* Sachtl. (Hymenoptera: Ichneumonidae) to pierce the puparia (Hagley et al. 1993). However, the puparial age of the tephritid is much greater than that of *M. domestica* (about 90 d versus about a week).

The present study suggests that in *S. endius*, ovipositing females would not affect their own or their offspring’s wear even if they preferentially drilled smaller younger hosts, which they do not (King 2000, 2002). This conclusion comes from the lack of evidence of wear, including no ovipositor wear even with large old hosts. However, effects of host size and age on puparial thickness and intractability may still affect a female’s fitness, by affecting the duration or success of her drilling through a puparium or of her offspring chewing out (King 1998, 2000, 2002).

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References Cited

- Broski, S. A. 2013.** The effects of host size and age on the fitness of *Spalangia endius* Walker (Hymenoptera: Pteromalidae). M. S. thesis, Northern Illinois University, DeKalb.
- Clissold, F. J., G. D. Sanson, J. Read, and S. J. Simpson. 2009.** Gross vs. net income: how plant toughness affects performance of an insect herbivore. *Ecology* 90: 3393-3405.
- Cribb, B. W., A. Stewart, H. Huang, R. Truss, B. Noller, R. Rasch, and M. P. Zalucki. 2008.** Unique zinc mass in mandibles separates drywood termites from other groups of termites. *Naturwissenschaften* 95: 433-441.
- Depieri, R. A., F. Siqueira, and A. R. Panizzi. 2010.** Aging and food source effects on mandibular stylets teeth wear of phytophagous stink bug (Heteroptera: Pentatomidae). *Neotrop. Entomol.* 39: 952-956.
- Dwivedi, J., and O. P. Agrawal. 1995.** Degradation of cuticle during larval-pupal and pupal-adult development of the housefly, *Musca domestica*. *Physiol. Entomol.* 20: 318-322.
- Fellowes, M. D. E., P. Masnata, A. R. Kraaijeveld, and H. C. J. Godfray. 1998.** Pupal parasitoid attack influences the relative fitness of *Drosophila* that have encapsulated larval parasitoids. *Ecol. Entomol.* 23: 281-284.
- Follett, P. A. 2009.** Puncture resistance in 'Sharwil' avocado to oriental fruit fly and Mediterranean fruit fly (Diptera: Tephritidae) oviposition. *J. Econ. Entomol.* 102: 921-926.
- Fritz, R. S., and E. L. Simms. 1992.** Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics, University of Chicago Press, Chicago, IL.
- Godfray, H. C. J. 1994.** Parasitoids, Princeton University Press, Princeton, NJ.
- Gross, P. 1993.** Insect behavioral and morphological defenses against parasitoids. *Ann. Rev. Entomol.* 38: 251-273.
- Hagley, E. A. C., A. R. Biggs, G. E. Timbers, and J. Coutu-Sundy. 1993.** Effect of age of the puparium of the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), on parasitism by *Phygadeuon wiesmanni* Sachtl. (Hymenoptera: Ichneumonidae). *Can. Entomol.* 125: 721-724.
- Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007.** Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol. Evol. Syst.* 8: 157-178.
- Hewitt, C. G. 1908.** The structure, development, and bionomics of the house-fly, *Musca domestica*, Linn. Part II.—The breeding habits, development, and the anatomy of the larva. *Q. J. Microsc. Sci.* 52: 495-545.
- Ibanez, S., S. Lavorel, S. Puijalon, and M. Moretti. 2013.** Herbivory mediated by coupling between biomechanical traits of plants and grasshoppers. *Funct. Ecol.* 27: 479-489.
- IBM Corp. Released 2012.** IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.
- Jervis, M. A., and N. A. C. Kidd. 1986.** Host-feeding strategies in hymenopteran parasitoids. *Biol. Rev.* 61: 395-434.
- Jones, S. R., and K. C. Kim. 1994.** Aculeus wear and oviposition in four species of Tephritidae (Diptera). *Ann. Entomol. Soc. Am.* 87: 104-107.
- Kikvidze, Z., and J. Moya-Laraño. 2008.** Unexpected failures of recommended tests in basic statistical analyses of ecological data. *Web Ecol.* 8: 67-73.
- King, B. H. 1988.** Sex ratio manipulation in response to host size by the parasitoid wasp *Spalangia cameroni*: a laboratory study. *Evolution* 42: 1190-1198.
- King, B. H. 1998.** Host age response in the parasitoid wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). *J. Insect Behav.* 11: 103-117.
- King, B. H. 2000.** Sex ratio and oviposition responses to host age and the fitness consequences to mother and offspring in the parasitoid wasp *Spalangia endius*. *Behav. Ecol. Sociobiol.* 48: 316-320.
- King, B. H. 2001.** Parasitization site on the host of the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae). *Environ. Entomol.* 30(2): 346-349.
- King, B. H. 2002.** Breeding strategies in females of the parasitoid wasp *Spalangia endius*: effects of mating status and body size. *J. Insect Behav.* 15: 181-193.
- King, P. E. 1962.** The muscular structure of the ovipositor and its mode of function in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Proc. Roy. Entomol. Soc., London, Ser. A* 37: 121-128.
- Kinjo, H., Y. Kunimi, T. Ban, and M. Nakai. 2013.** Oviposition efficacy of *Drosophila suzukii* (Diptera: Drosophilidae) on different cultivars of blueberry. *J. Econ. Entomol.* 106: 1767-1771.

- Kraaijeveld, A. R., and H. C. J. Godfray. 2003.** Potential life-history costs of parasitoid avoidance in *Drosophila melanogaster*. *Evol. Ecol. Res.* 5: 1251-1261.
- Lin, P., and W. Q. Wang. 2001.** Changes in the leaf composition, leaf mass and leaf area during leaf senescence in three species of mangroves. *Ecol. Eng.* 16: 415-424.
- Massey, F. P., and S. E. Hartley. 2009.** Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *J. Anim. Ecol.* 78: 281-291.
- Michon, G. P. 2013.** Final answers. <http://www.numericana.com/answer/ellipsoid.htm>
- Morris, R. J., and M. D. E. Fellowes. 2002.** Learning and natal host influence host preference, handling time and sex allocation behaviour in a pupal parasitoid. *Behav. Ecol. Sociobiol.* 51: 386-393.
- Motomura, H., N. Mita, and M. Suzuki. 2002.** Silica accumulation in long-lived leaves of *Sasa veitchii* (Carriere) Rehder (Poaceae-Bambusoideae). *Ann. Bot. London* 90: 149-152.
- Quicke, D. L. J., M. G. Fitton, J. R. Tunstead, S. N. Ingram, and P. V. Gaitens. 1994.** Ovipositor structure and relationships within the Hymenoptera, with special reference to the Ichneumonoidea. *J. Nat. Hist.* 28: 635-682.
- Quicke, D. L. J., P. Wyeth, J. D. Fawke, H. H. Basibuyuk, and J. F. V. Vincent. 1998.** Manganese and zinc in the ovipositors and mandibles of hymenopterous insects. *Zool. J. Linn. Soc.* 124: 387-396.
- Quicke, D. L. J., J. Palmer-Wilson, A. Burrough, and G. R. Broad. 2004.** Discovery of calcium enrichment in cutting teeth of parasitic wasp ovipositors (Hymenoptera: Ichneumonoidea). *Afr. Entomol.* 12: 259-264.
- Raupp, M. J. 1985.** The effects of leaf toughness on mandibular wear of the leaf beetle *Plagioderia versicolora*. *Ecol. Entomol.* 10: 73-79.
- Rivers, D. B. 2004.** Evaluation of host responses to envenomation as a means to assess ectoparasitic pteromalid wasp's potential for controlling manure-breeding flies. *Biol. Control* 30: 181-192.
- Roitberg, B., D. Gillespie, D. Quiring, C. Alma, W. Jenner, J. Perry, J. Peterson, M. Salomon, and S. VanLaerhoven. 2005.** The cost of being an omnivore: mandible wear from plant feeding in a true bug. *Naturwissenschaften* 92: 431-434.
- Roseland, C. R., M. J. Grodowitz, K. J. Kramer, T. L. Hopkins, and A. B. Broce. 1985.** Stabilization of mineralized and sclerotized puparial cuticle of muscid flies. *Insect Biochem.* 15: 521-528.
- Rueda, L. M., and R. C. Axtell. 1985.** Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure, Technical Bulletin 278: 1-88. North Carolina Agricultural Research Service, North Carolina State University, Raleigh.
- Schofield, R. M. S., M. H. Nesson, and K. A. Richardson. 2002.** Tooth hardness increases with zinc-content in mandibles of young adult leaf-cutter ants. *Naturwissenschaften* 89: 579-583.
- Schofield, R. M. S., K. D. Emmett, J. C. Niedbala, and M. H. Nesson. 2011.** Leaf-cutter ants with worn mandibles cut half as fast, spend twice the energy, and tend to carry instead of cut. *Behav. Ecol. Sociobiol.* 65: 969-982.
- Takasu, K., and Y. Hirose. 1991.** The parasitoid *Ooencyrtus nezarae* (Hymenoptera, Encyrtidae) prefers hosts parasitized by conspecifics over unparasitized hosts. *Oecologia* 87: 319-323.
- Tormos, J., F. Beitia, E. A. Böckmann, and J. D. Asís. 2009.** The preimaginal stages and development of *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) on *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Micron* 40: 646-658.
- Vincent, J. F. V., and S. Ablett. 1987.** Hydration and tanning in insect cuticle. *J. Insect Physiol.* 33: 973-979.
- Vincent, J. F. V., and J. E. Hillerton. 1979.** The tanning of insect cuticle -- a critical review and a revised mechanism. *J. Insect Physiol.* 25: 653-658.
- Zar, J. H. 2010.** Biostatistical analysis, 5th ed. Prentice Hall, Upper Saddle River, NJ.

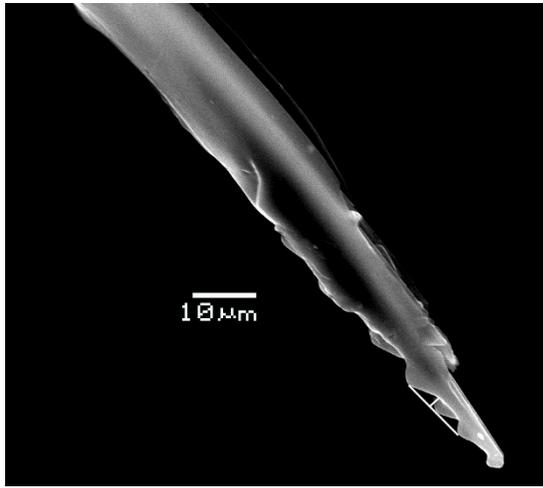


Fig. 1. Distal end of the two lower valves of an *S. endius* ovipositor. The index of wear was calculated as the distance of an imaginary line between the tips of the second and third serrations, divided by the depth from that line to the lowest point between those serrations, as indicated on the figure.

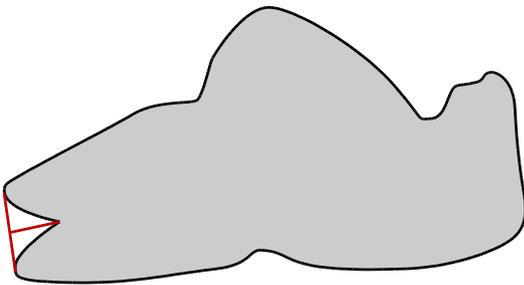


Fig. 2. Left mandible of a female *S. endius*. The index of wear was calculated as the distance between the tips of the two incisors, divided by the depth from that line to the lowest point between the incisors, as indicated on the figure.

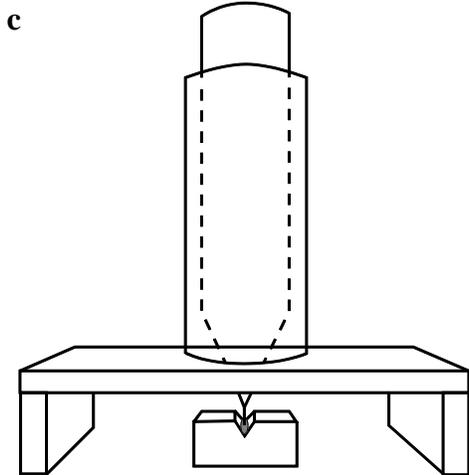


Fig. 3. Penetrometer used to find the force required to penetrate the puparium.

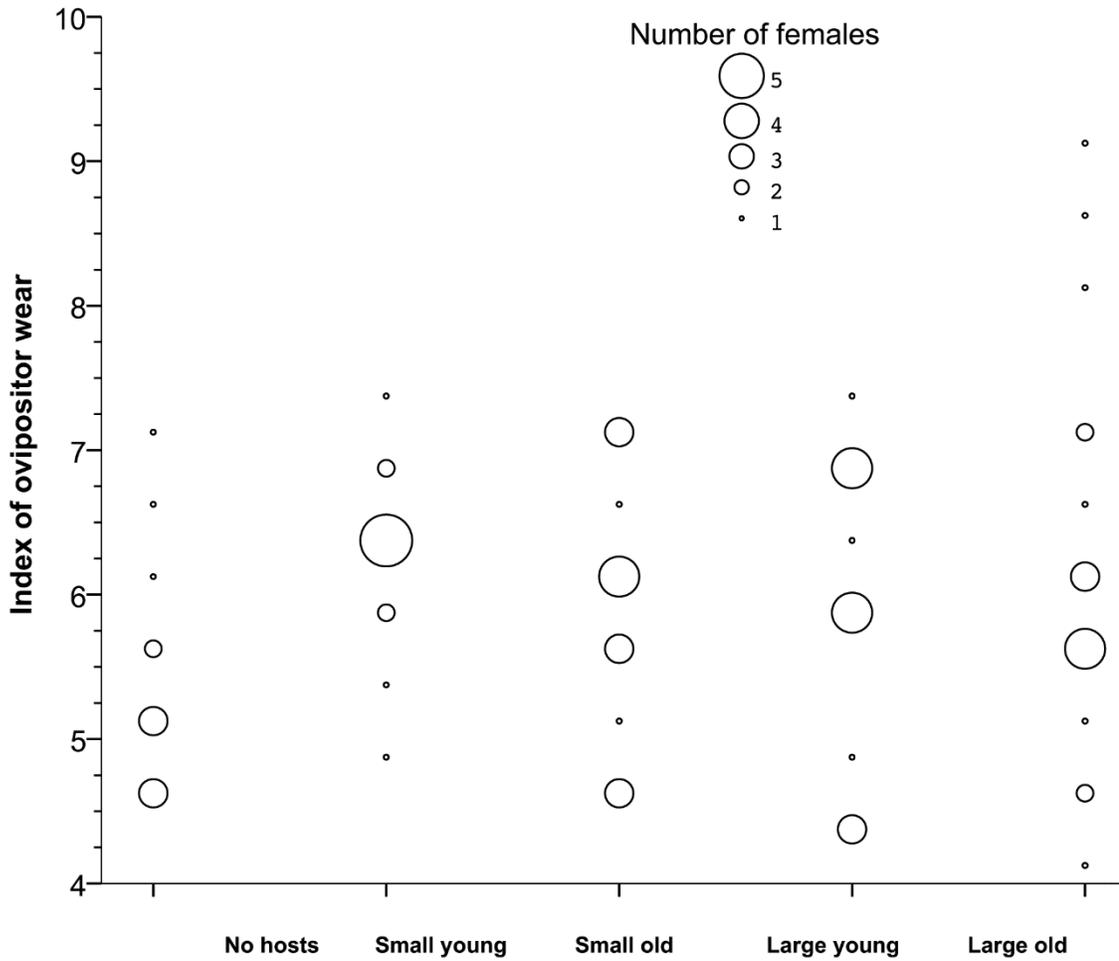


Fig. 4. Index of ovipositor wear for the naïve control in which females did not receive hosts and the four experienced treatments in which females received hosts. A higher index indicates a higher degree of wear.

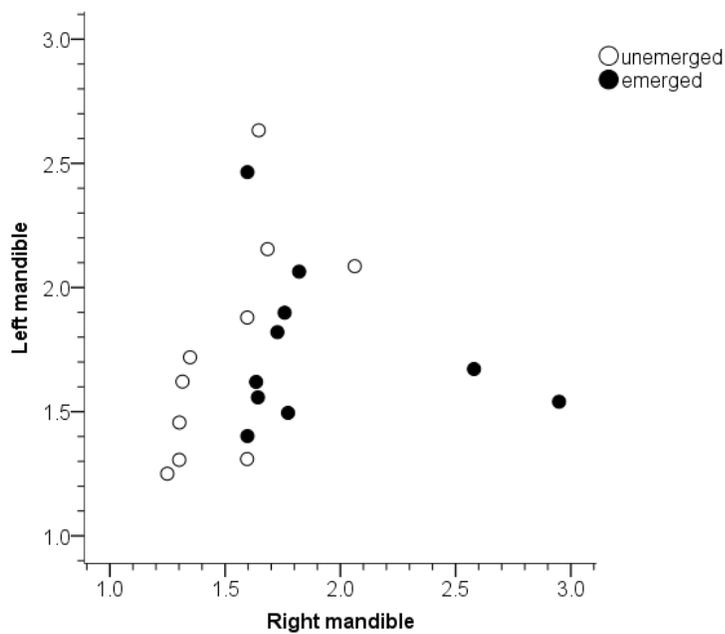


Fig. 5. Index of wear of left and right mandibles of emerged and unemerged females (n = 10 females per treatment). A higher index indicates a higher degree of wear.

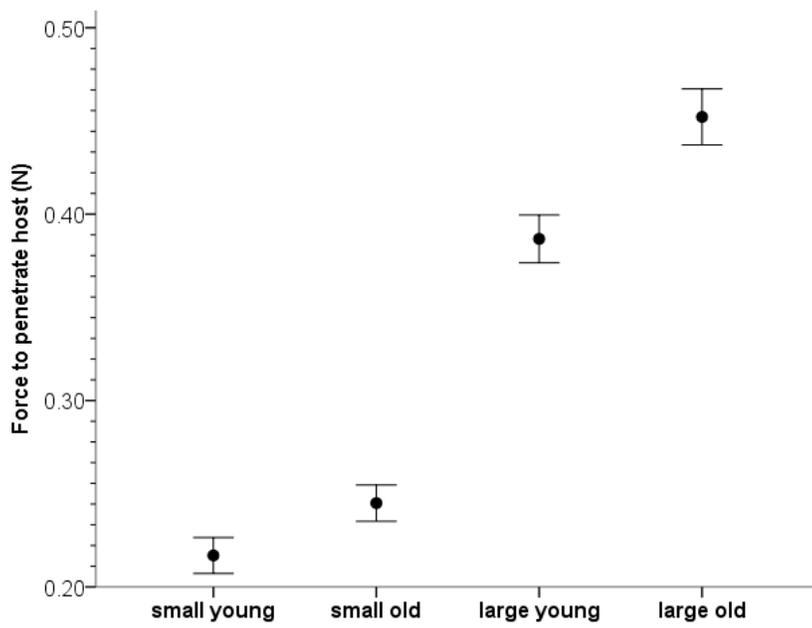


Fig. 6. Mean \pm SEM force required to penetrate the puparia of hosts of varied size and age (n = 20 hosts per size – age treatment).

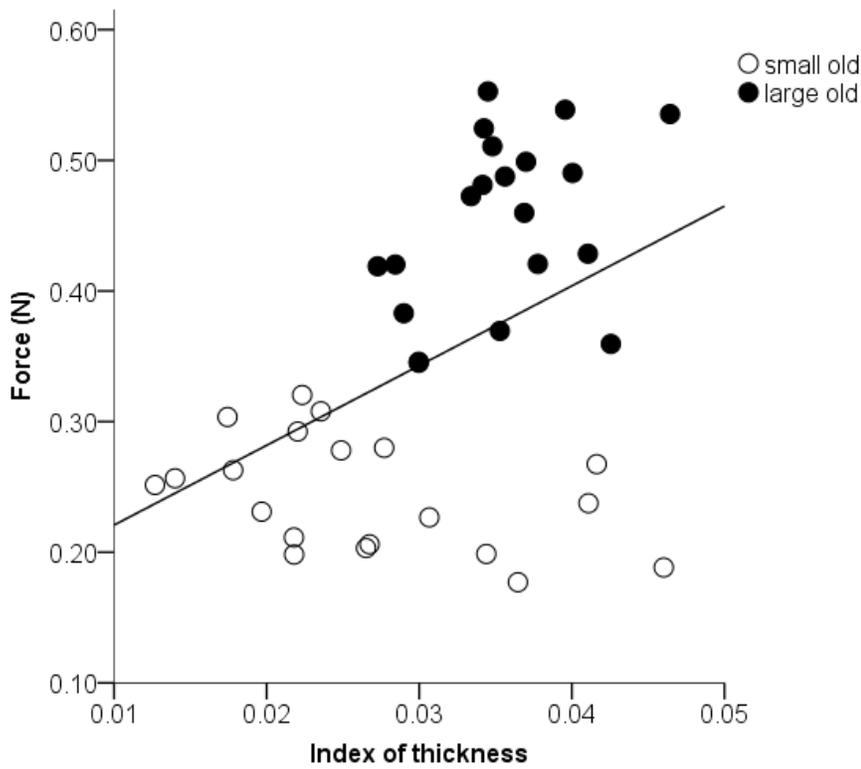


Fig. 7. Relationship between the puparium's index of thickness and the force required to penetrate it among old hosts.