



Juvenile hormone manipulation affects female reproductive status and aggressiveness in a non-social parasitoid wasp

Anthony G.E. Mathiron, Ryan Earley, Marlène Goubault

► To cite this version:

Anthony G.E. Mathiron, Ryan Earley, Marlène Goubault. Juvenile hormone manipulation affects female reproductive status and aggressiveness in a non-social parasitoid wasp. *General and Comparative Endocrinology*, 2019, 274, pp.80-86. 10.1016/j.ygcen.2019.01.006 . hal-02140550

HAL Id: hal-02140550

<https://hal.science/hal-02140550>

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Juvenile Hormone manipulation affects female reproductive status and aggressiveness in a non-social parasitoid wasp

Anthony G.E. Mathiron¹, Ryan L. Earley² and Marlène Goubault¹

¹Institut de Recherche sur la Biologie de l’Insecte, UMR 7261 CNRS - Université de Tours, Parc Grandmont, 37200 Tours, France

²Department of Biological Sciences, The University of Alabama, 300 Hackberry Lane, Box 870344, Tuscaloosa, AL 35487, United States

Author for correspondence:

Anthony Mathiron

Telephone number: +33 02 47 36 73 55

e-mail: anthony.mathiron@sfr.fr

Abstract. In vertebrates, titers of androgens such as testosterone are known to upregulate aggressive behaviors associated with reproduction. In insects, juvenile hormone (JH) is a good candidate for studying the flexibility of insect endocrine responses because it has important effects on both reproductive processes and behavior. JH has a gonadotropic effect across a broad range of insect species, increasing ovarian development in females, and may have a role in the regulation of aggressive behavior during competition. In Hymenoptera, the functions of JH have been studied in facultatively eusocial species such as polistine wasps, bumblebees, ants and bees. Surprisingly, no work has yet focused on the relationship between JH, reproduction and aggressiveness in a non-social Hymenoptera, although it may help to understand how JH actions have evolved across taxa with different degrees of sociality. Here, we explored how JH treatment influenced: i) female reproductive status, and ii) the intensity (aggressiveness) and resolution of conflict, in *Eupelmus vuilleti* (Hymenoptera: Eupelmidae), a solitary ectoparasitoid wasp in which females fight over hosts.

We demonstrated that intra-abdominal injections of JH increased the number of mature eggs in females after 24h. In addition, the number of aggressive behaviors displayed by females was affected by the interaction between JH treatment and the number of mature eggs in their abdomen, but mature egg load alone predicted the outcome of staged contests. Wasps were more aggressive when they had more ready-to-lay eggs, with this effect being stronger when females were injected with JH. Moreover, females won more frequently when they had higher mature egg load. Our results highlight how JH affects egg maturation and aggressive behaviors in *Eupelmus vuilleti* females. To our knowledge, this is the first study showing that hormone manipulation can modulate females' reproduction status and behavior during intraspecific competition over hosts in a non-social hymenopteran parasitoid.

Keywords: juvenile hormone, reproductive maturation, aggressive behaviors, conflict resolution

1. Introduction

As gaining access to resources brings fitness benefits to individuals, both vertebrate and invertebrate animals commonly exhibit aggressive behaviors during conflicts over such resources (Collias, 1944; Breed and Bell, 1983; Briffa and Hardy, 2013). For example, in parasitoid insects, hosts represent an indivisible resource over which females compete to lay their eggs, and physical interactions between females for host access have been observed in various parasitoid species (Hughes et al., 1994; Petersen and Hardy, 1996; Field and Calbert, 1999; Goubault et al., 2007; Mohamad et al., 2010; Mathiron et al., 2018). In many animal taxa, aggressive conflicts have also been observed between individuals for sexual partners (West-Eberhard, 1979; Schwagmeyer and Woontner, 1985; Simmons, 1986), food (Barton, 1993; Vogel, 2005), territories (Rowland, 1989; Festa-Bianchet et al., 1990), and dominance status (Chase, 1974). However, being constantly aggressive can be costly, as potential physical injuries can lead to death (Palombit, 1993; Innocent et al., 2011), and decreased vigilance can increase predation risk (Jakobsson et al., 1995; Díaz-Uriarte,

1999). Animals should therefore evaluate the costs and benefits of exhibiting agonistic behaviors and then behave appropriately (Maynard-Smith and Price, 1973).

Endocrine regulation is one important way that animals can match behavioral and physiological responses to their current environment (Tibbetts and Crocker, 2014). In vertebrates, testosterone is generally involved in the modulation of aggressive behaviors during conflicts associated with reproduction, such as the establishment and maintenance of breeding territories, mate access, and offspring defense (Wingfield, 1984). For example, Salvador et al. (1996) showed that testosterone supplementation in large males of the territorial lizard *Pseudommodon algirus* increased the number of chasing behaviors toward conspecific intruders. Moreover, during mate choice, female zebra finches treated with testosterone were more aggressive toward conspecific females than control individuals (Adkins-Regan, 1999). Whilst well- described in vertebrates, the endocrine basis of behavior is still relatively unknown in insects (Zera, 2007; Tibbetts et al., 2014). Because vertebrates and insects have similar behaviors but different endocrine systems (Nijhout, 1994; Adkins-Regan, 2005), exploring how hormones modulate insects behaviors can therefore provide insight into the evolution of endocrine regulation of behavior (Emlen and Nijhout, 2000).

In insects, juvenile hormone (JH) is a good candidate for studying the flexibility of endocrine responses to intraspecific conflict because it has important effects on both reproductive processes and behavior. Together with nutrition (see the review of Wheeler, 1996), JH plays an important and diverse role in reproduction in many adult insect species by regulating vitellogenesis in the fat body and vitellogenin uptake by the ovaries (reviewed by Tobe and Stay, 1985; Nijhout, 1994; Robinson and Vargo, 1997; Hartfelder, 2000). In Hymenoptera, JH is involved in the regulation of ovarian development in the facultatively eusocial sweat bee *Megalopta genalis* (Smith et al., 2013) and in primitively social species like polistine wasps (Barth et al., 1975), halictine bees (Bell, 1973) and bumblebees (Bloch et al., 2000; Amsalem et al., 2014). However, in the highly social honeybee *Apis mellifera*, JH is apparently involved in the regulation of division of labor but has no clear role in the

76 regulation of reproduction (reviewed in Robinson, 1992; Robinson and Vargo, 1997). In many
77 eusocial hymenopterans, JH is also associated with displays of behaviors often judged to be
78 aggressive (Tibbetts and Crocker, 2014). For example, in the epiponine wasp *Polybia micans*, JH
79 may fuel aggressive behaviors of single queens heading a colony (Kelstrup et al. 2014a). Similar
80 observations have been made in paper wasps, in which high JH titers are associated with dominance
81 behaviors (Röseler et al., 1980, 1984; Giray et al., 2005; Tibbetts and Huang, 2010; Tibbetts et al.,
82 2013; Kelstrup et al. 2016; but see Kelstrup et al., 2015). In honeybees, JH titers are higher in more
83 aggressive workers and JH treatments cause honeybees to exhibit more frequent guarding behaviors
84 (Sasagawa et al., 1989; Pearce et al., 2001). However, JH has no effect on aggression in the swarm-
85 founding epiponine wasp *Synoecca surinama* (Kelstrup et al., 2014b) and may even be negatively
86 correlated with aggression in the queenless ponerine ant, *Streblognathus peetersi* (Brent et al., 2006).
87 Surprisingly, no work has yet focused on the relationship between JH, reproduction, and
88 aggressiveness in strict non-social Hymenoptera species, although it may help to understand how JH
89 actions have evolved across insect taxa with different degrees of sociality.

90 In the present study, we investigated how JH treatment influences: i) female reproductive
91 status, and ii) aggressive behaviors exhibited during contests over host access as well as the outcome
92 of such contests, using *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae). *Eupelmus vuilleti* is
93 a solitary ectoparasitoid (i.e. only one juvenile can develop per host, with supernumerary offspring
94 being killed during larval competition). Females parasitize and feed upon larvae and pupae of
95 *Callosobruchus maculatus* (Fab) (Coleoptera: Bruchidae), which infest cowpea seeds, *Vigna*
96 *unguiculata* (L.) Walp (Fabaceae; Terrasse and Rojas-Rousse, 1986). When several females are
97 simultaneously present on a host patch, they tend to protect the host that they exploit by displaying
98 aggressive behaviors towards conspecific competitors (Mohamad et al., 2010; Mathiron et al., 2018).
99 Previous studies have investigated the role of different factors (e.g., egg load, host density and host
100 quality) on aggressiveness and contest outcomes in *E. vuilleti* (Mohamad et al., 2010, 2012, 2013;

101 Mathiron et al., 2018), but nothing is known about how hormones modulate aggressiveness during
102 competition for host access.

103 While measuring variation in hormone titers is an important first step to understand the
104 endocrine basis of phenotypic plasticity (Zera, 2007), one method that allows for understanding
105 individual responses to hormonal change is to administer a similar amount of hormone to numerous
106 individuals and, subsequently, to test how hormone manipulations affect the phenotype (Tibbetts and
107 Izzo, 2009). As such, manipulation of JH is one of the most common methods employed to
108 understand its mode of action and functions in insects (Sláma, 1971; Ramaseshadri et al., 2012). A
109 first experiment was therefore designed to determine if JH treatment affected female reproductive
110 status. As for many other insect species, we expected JH to have a gonadotropic effect in females,
111 i.e. that wasps treated with JH would have more ready-to-lay eggs than control females (i.e. treated
112 with vehicle). In a second experiment, we studied whether aggressiveness and resolution of conflicts
113 between females were influenced by a JH treatment. We expected that individuals treated with JH
114 would display more aggressive behaviors and would ultimately be more likely to win contests for
115 hosts relative to control females.

116

117 **2. Material and Methods**

118 *2.1. Laboratory breeding*

119 In 2007, *E. vuilleti* emerged from cowpea seeds collected in Togo (West Africa) and infested
120 by the bruchid beetle *Callosobruchus maculatus*. Parasitoids were then bred in the laboratory (IRBI,
121 University of Tours, France) on larval and pupal stages of *C. maculatus*, according to the
122 methodology described by Jaloux et al. (2004). All experimental procedures were carried out in a
123 climate room at 30°C and 12:12 h light: dark. Cowpea seeds were dissected to collect hosts for *E.*
124 *vuilleti*, which were then individually placed in a standard transparent gelatin capsule (length: 2 cm,
125 diameter: 0.6 cm). This system mimics a cowpea seed without significantly altering the oviposition

126 behavior of females, and facilitates observation of the number of eggs laid by females (Gauthier and
127 Monge, 1999). Previous studies have shown that after a period of acclimation, parasitoid females
128 behave in the same manner to the presence of hosts inside seeds and capsules (Gauthier and Monge,
129 1999; Jaloux et al., 2004), although the number of eggs laid on hosts is slightly lower inside capsules
130 (Damiens et al., 2001).

131 Females used in the following experiments emerged in isolation, which prevented them from
132 having any previous experience of oviposition or competition. We placed them individually in petri
133 dishes (diameter: 8.5 cm; height: 2.7 cm), and provided cotton soaked in water and one pupa of *C.*
134 *maculatus* located in gelatin capsule that was renewed each day preceding the experiments. *E.*
135 *vuilleti* is a synovigenic parasitoid species: emerging females possess a few mature eggs and they
136 mature additional eggs throughout their adult life. Oviposition activity increases over time and peaks
137 three days after emergence (Jaloux et al., 2004). To stimulate oogenesis (Terrasse and Rojas-Rousse,
138 1986), we also gave one male to females during the first day.

139 2.2. Experiment 1: Effect of JH on females' reproductive status

140 To investigate the influence of JH treatment on female reproductive status, two-day old wasps
141 were randomly assigned to one of the five following treatments: they received either 0.04 μ L of
142 acetone (N=36) or 0.04 μ L of JHIII (purchased from Sigma-Aldrich, Inc.) dissolved in acetone at
143 four different concentrations (8, 16, 32 or 64 pg/ μ l; N=35, N=39, N=39, N=37, respectively). The
144 final volume was established based on preliminary tests showing that mean haemolymph volume in
145 *E. vuilleti* females is 0.175 μ l (but this volume may vary depending on body mass, physiological
146 state or environmental conditions; Borde et al., unpublished data). Immediately after being
147 immobilized on a petri dish that was cooled by ice, wasps were injected into the abdomen through
148 the intersegmental membrane using the nanoliter injector Nanoject III (Drummond Scientific
149 Company). Females were then individually placed in a petri dish and were frozen at -20°C either 4h
150 (N=20 for injections of 0, 8, 16, 32 pg/ μ l and N=18 for injection of 64 pg/ μ l) or 24h (N=16 for

151 injection of 0 pg/ μ l, N=15 for injection of 8 pg/ μ l, and N=19 for injections of 16, 32 and 64 pg/ μ l)
152 after injection. Females were deprived of hosts so that no mature eggs were laid during this period.
153 Finally, all females were weighed using an electronic balance (Ohaus Discovery® model, accuracy:
154 0.01mg) and then dissected to count mature (i.e. ready-to-lay) oocytes in their abdomen.

155

156 2.3. *Experiment 2: Effect of JH on females' aggressiveness and contests outcome*

157 To investigate the influence of JH manipulation on female aggressive behavior, three-days
158 old wasps were randomly assigned to two different injection treatments: females injected with either
159 0.04 μ L of JHIII diluted in acetone (concentration = 32 pg/ μ l) or 0.04 μ L of acetone alone. Wasps
160 were marked on the dorsal part of their thorax with a dot of bright yellow or bright red acrylic paint
161 the day before behavioral observations to allow individual identification during contests. Paint color
162 did not affect contest outcomes (wasps painted red won 17 of 25 contests; binomial test: $P=0.11$).
163 Females were deprived of hosts for at least 2h before the start of the contest so that they were more
164 ready to oviposit. At the beginning of the observation, two wasps were simultaneously introduced
165 into an apparatus consisting of a plastic block made of three chambers linked by a narrow channel
166 (Petersen and Hardy, 1996; Fig. 1.).

167

168

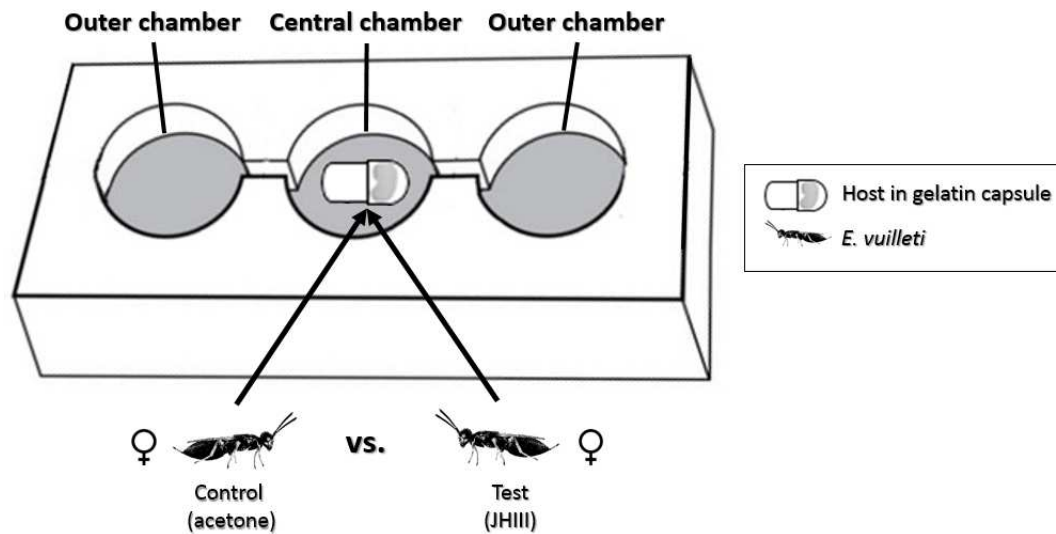


Figure 1: Experimental apparatus (adapted from Peterson & Hardy, 1996). Three chambers (diameter = 1.8 cm, depth = 0.6 cm) linked by a narrow channel (width = 0.1 cm) were made in a plastic block, decked with Plexiglas. At the start of experiments, two *E. vuilleti* females (one control and one test female) were simultaneously introduced into the central chamber already containing a 4th instar larva of *C. maculatus* within a gelatin capsule mimicking a seed. Wasp behavior was observed from above the contest arena.

All contests were run between one control female (i.e., injected with acetone only) and one test female (i.e., injected with JHIII), challenging for a 4th instar larva of *C. maculatus* located in gelatin capsule and previously placed in the central chamber of the contest arena (Fig.1.). Mathiron et al. (2018) showed that females with previous oviposition experience on pupae were more motivated to gain access to a 4th instar larva. Contests occurred between females of the same age (3 days old), marked with different colors and visually matched for size; post-experiment data analysis confirmed that contestants of the same dyad did not differ significantly in body mass (Wilcoxon signed-ranks test: $T=147.5$, $N=25$, $P=0.7$). Both females could freely move throughout the entire contest arena. Observations lasted 1h or were stopped when: i) one female pushed her opponent out of the central chamber, ii) one female exited the central chamber for at least 2 min, or iii) neither female touched the host nor displayed aggressive interactions for at least 2 min.

187 During all tests, we recorded oviposition and aggressive behaviors displayed by each female.
188 Upon detection of a conspecific (females raised their antennae in the direction of their opponent),
189 females frequently interrupted their behavioral oviposition sequence (Mohamad et al., 2010). They
190 either simply kick their opponent with their legs without taking their ovipositor out of the capsule
191 (defensive behavior) or can escalate to a full attack, in which case one female generally chases her
192 opponent away from the capsule, hits her with her head or mounts her (Mathiron et al., 2018).
193 Chasing, hitting and mounting can lead to the loser leaving the central chamber of the arena (8/25
194 loser females left the central chamber after an opponent attack during this experiment). Only these
195 behaviors, collectively termed as ‘attacks’, were therefore considered in the rest of the study. When
196 both females of a dyad stayed in the central chamber during the whole time of observation, the
197 winning female was determined as the one exhibiting oviposition behaviors.

198 Wasps were immediately frozen at -20°C after the experiments, weighed using an electronic
199 balance (Ohaus Discovery® model, accuracy: 0.01mg), and dissected to count the number of mature
200 and non-mature eggs in their abdomen. Finally, after each contest, we counted the number of eggs
201 laid on the host by the winning female to determine their mature egg load before the contests (i.e.,
202 initial mature egg load).

203 *2.4. Statistical analysis*

204 We performed data analyses with the software Rstudio (1.0.143 version – © 2009-2016
205 RStudio, Inc.), using $\alpha=0.05$. Our general approach was to use, when possible, parametric analyses in
206 which the assumed distribution of residuals was matched to the data rather than transforming data to
207 fit standard assumptions (Wilson and Hardy, 2002; Briffa et al., 2013). However, we used non-
208 parametric tests when parametric conditions were not verified.

209 Experiment 1: We first investigated whether the mature egg load of females differed
210 significantly between JHIII treatments, 4h after injection, by performing a general linear model
211 (GLM) assuming a Poisson distribution of errors. We then ran a linear model (LM) and a polynomial

212 linear model (PLM) to explore the effect of JHIII treatments on the number of mature eggs 24h after
213 injection, and we compared the linear and polynomial models to determine which one provided a
214 better fit.

215 Experiment 2: We first verified that initial mature egg load did not differ between JHIII
216 females (mean egg load \pm S.E.: 6.2 ± 0.7) and control females (mean egg load \pm S.E.: 5 ± 0.5 ;
217 Student test: $t_{25}=-1.41$, $P=0.16$). We then performed a generalized linear mixed model (GLMM) with
218 a Poisson error distribution to explore the influence of contestants' initial mature egg load and
219 injection treatment on the number of attacks (calculated as the sum of chasing, hitting, and mounting
220 behaviors) displayed by females, with contest identity as a random factor. Lastly, we conducted a
221 GLMM assuming a binomial error distribution to explore the influence of initial mature egg load of
222 females and injection treatments on contest outcomes, with contest identity as a random factor. To
223 run this analysis, we defined contest outcome as a binary response: 0 = the female lost and 1 = the
224 female won.

225 **3. Results**

226 *3.1. Experiment 1: Effect of JH on female reproductive status*

227 The number of mature eggs in females did not vary across treatments 4h after
228 injections (GLM: $\chi^2=0.006$, $df=1$, $P=0.94$; Fig.2a). However, 24h after injections, mature egg
229 load was influenced significantly by treatment (LM: $F_{1,86}=4.05$, $P=0.047$, $R^2=0.335$).
230 Moreover, we found that the polynomial model fitted better than the linear model, with a
231 dose-dependent effect of JHIII injection on egg maturation reaching a plateau at 64 pg/ μ L
232 (PLM: $F_{1,85}=4.05$, $P=0.047$, $R^2=0.365$; Fig.2b).

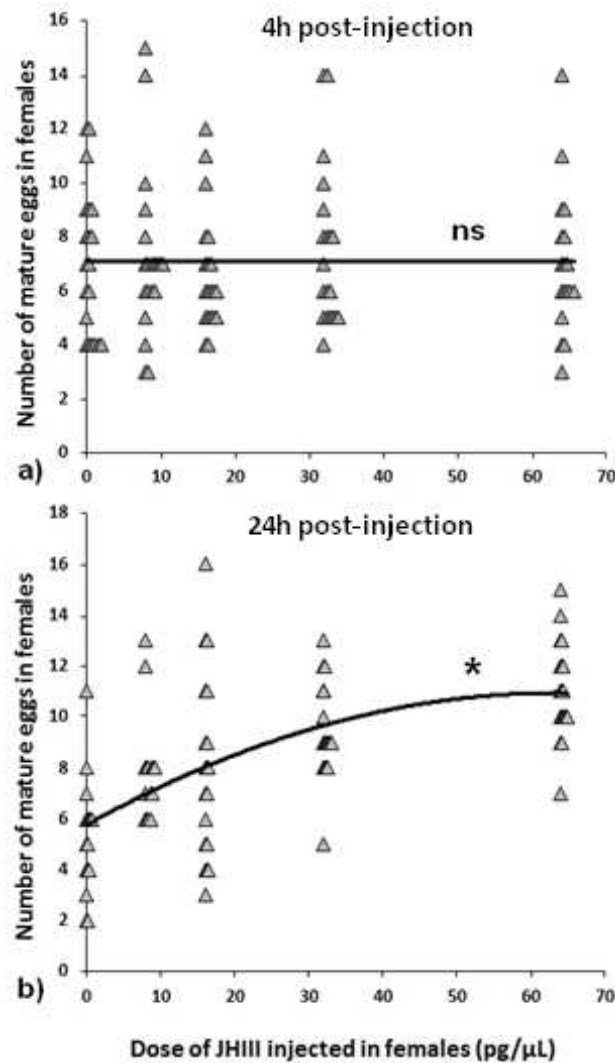


Figure 2: Number of mature eggs of females injected with different doses of JHIII. **a)** 4h after injections, **b)** 24h after injections. ns: non-significant; *: P < 0.05. Data points have been horizontally displaced from their binary positions to show the numbers of observations.

3.2. Experiment 2: Effect of JH on female aggressiveness and contests outcome

The number of attacks displayed by females was influenced significantly by the interaction between female egg load and injection treatment (Table 1). Wasps attacked significantly more during a contest when they had more ready-to-lay eggs, and this effect was stronger when females were injected with JHIII compared to control females injected with acetone alone (Table 1, Fig. 3). Effect of female egg load x injection treatment interaction

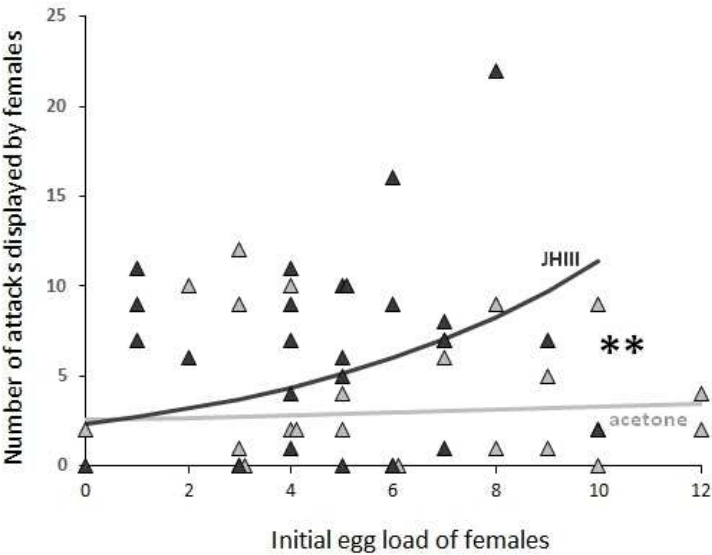
244 was still significant when removing data from the contest with a JHIII female displaying 22 attacks
 245 and her control opponent displaying 2 attacks (GLMM: $\chi^2=4.87$, $df=1$, $P=0.03$).

246

247 Table 1. Effect of initial egg load difference and injection on female agonistic behaviors.

Factors affecting female aggressiveness	df	χ^2	P
Egg load	1	4.81	< 0.05
Injection	1	20.34	< 0.001
Egg load x Injection	1	7.09	0.008

248 *P*-values of significant explanatory variables are shown in bold font



249

250 Figure 3. a) Number of attacks displayed by females treated with acetone alone (light data points, light gray line; N=25)
 251 or with JHIII (dark data points, dark curve; N=25) when fighting for a 4th instar larva of *C. maculatus*. **: $P < 0.01$. Data
 252 points have been horizontally displaced from their binary positions to show the numbers of observations. Statistical
 253 analyses showed that effect of ‘Egg load x Injection’ interaction was still significant when removing data from the
 254 contest with a JHIII female displaying 22 attacks and her control opponent displaying 2 attacks (GLMM: $\chi^2=4.87$, $df=1$,
 255 $P=0.03$).

256

257 The probability of a female winning a contest was not influenced significantly by
 258 either the interaction between her initial mature egg load and injection, or the effect of

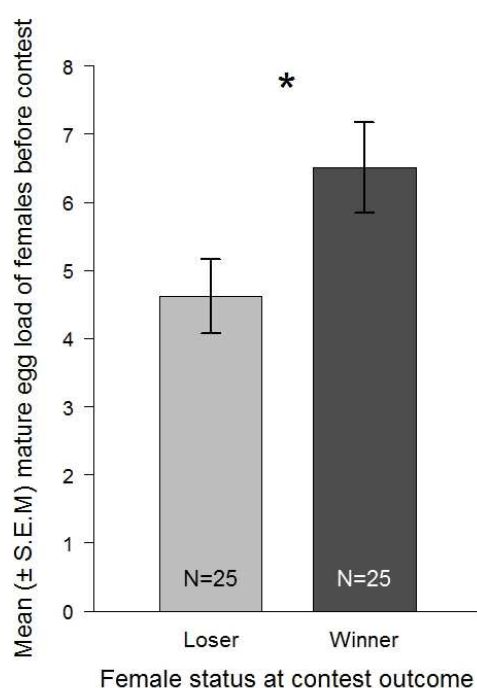
259 injection alone (Table 2). However, females had higher probabilities of winning contest
 260 when they had more ready-to-lay-eggs before contests (Table 2, Fig. 4).

261

262 Table 2. Effect of initial egg load and injection on the winning probability of females.

Factors affecting contest outcomes	df	χ^2	P
Egg load	1	4.62	0.03
Injection	1	0.54	0.46
Egg load x Injection	1	0.22	0.64

263 *P*-values of significant explanatory variables are shown in bold font



264

265 Figure 4. Mean (± S.E.M) number of mature eggs in females before the contest (i.e. initial egg load) and their winning
 266 status at contest outcome. *: $P < 0.05$. Sample sizes are shown as text within each histogram bar.

267

268 4. Discussion

269 The purpose of our study was to test the effect of JH manipulation on female
 270 reproductive status, aggressiveness and conflict resolution in the solitary ectoparasitoid *E*.

271 *vuilleti*. By measuring how JHIII injections affect oocyte maturation, we showed that JHIII
272 did not influence mature egg load after 4h, but had a dose-dependent gonadotropic effect in
273 females 24h post-injection. Moreover, JHIII injections and egg load interacted to affect
274 female aggressiveness during competition for host access.

275 4.1. Effect of JH on females' reproductive status

276 In our first experiment, the reproductive status of the wasps varied after 24h according to the
277 amount of JHIII injected in females: wasps injected with JHIII had more ready-to-lay eggs than those
278 injected only with acetone. This result therefore suggests that JH has a gonadotropic effect in *E.*
279 *vuilleti* females, and was expected given that JH plays an important and diverse role in adult
280 reproduction of many insect species by regulating vitellogenesis in the fat body and vitellogenin
281 uptake by the ovaries (reviewed by Tobe and Stay, 1985; Nijhout, 1994; Robinson and Vargo, 1997;
282 Hartfelder, 2000). In Hymenoptera, JH is involved in the regulation of ovarian development in the
283 facultatively eusocial sweat bee *Megalopta genalis* (Smith et al., 2013), primitively social polistine
284 wasps (Barth et al., 1975), halictine bees (Bell, 1973) and bumblebees (Bloch et al. 2000), but not in
285 the highly social honeybee *Apis mellifera* (reviewed in Robinson, 1992; Robinson and Vargo, 1997).
286 Because egg maturation was affected by JH in a dose-dependent way, we can reject the hypothesis
287 that the physiological response to injection was an artefact related to activation of another signaling
288 pathway such as the stress response. Finally, we found no difference in mature egg load between
289 control and JHIII treated females 4h after injections. This is consistent with what we found in the
290 second experiment, where contestants had the same number of mature eggs before contests, whether
291 they were injected with JHIII or with vehicle alone. Our results therefore suggest that the egg
292 maturation response to JH injection may happen only after 4h post-injection. Decoupling the effect
293 of ovarian maturation and JH treatment on aggressive interactions is sometimes difficult (see Barth et
294 al., 1975 for an example). Observing *E. vuilleti* females fight over hosts between 1h and 4h post-

295 injection permitted us to disentangle the effects of JHIII and mature egg load on aggressiveness and
296 contest outcomes.

297 4.2. Effect of JH and Subjective Resource Value on female competition

298 In our second experiment, we investigated the role of JH injection and mature egg
299 load on female aggressiveness and contest outcome. Our results support the idea that
300 reproductive state influences behavioral decisions during fights (Neat et al., 1998; Stokkebo and
301 Hardy, 2000; Arnott and Elwood, 2008). As previously demonstrated in *E. vuilleti* (Mohamad et al.,
302 2010; Mathiron et al., 2018), we found that more gravid females (i.e., females with more mature
303 eggs) displayed more attacks and had a higher probability of winning contests. This is also
304 consistent with game theoretical predictions that contest outcome is influenced by differences in the
305 value that opponents place in the resource ('Subjective Resource Value', SRV; Maynard-Smith and
306 Parker, 1976; Hammerstein, 1981; Enquist and Leimar, 1987). For instance, parasitoid females with
307 more developed reproductive tissues are predicted to place a greater value on hosts: they
308 are more prepared to oviposit and should therefore be more motivated to fight and have a
309 higher probability of winning contests (Stokkebo and Hardy, 2000; Brown et al., 2006, 2007;
310 Dissanayake et al., 2009). We therefore added evidence that the initial egg load as a component of
311 SRV plays a main role in conflict resolution in *E. vuilleti*.

312 More interestingly, we found an interaction between JHIII injection and mature egg load on
313 the number of aggressive behaviors displayed by females. We observed that the effect of initial egg
314 load on aggressiveness was more pronounced when contestants were injected with JHIII. We
315 therefore suggest that JHIII promoted female aggressiveness during fights for host access. This
316 agrees with previous studies exploring the role of JH on agonistic behaviors in insects (Tibbetts and
317 Crocker, 2014). Topical application of methoprene (a JH analog) induced strong reactions to
318 confrontation (i.e., aggression/flight behavior) in individuals of *Schistocerca gregaria* and *Locusta*
319 *migratoria* (Wiesel et al., 1996). Scott (2006) made the same observation in *Nicrophorus orbicollis*

320 and *Nicrophorus tomentosus*: methoprene application significantly increased the number of injuries
321 from aggressive interactions in males and females in the absence of a resource. Moreover, in the
322 cockroach *Nauphoeta cinerea*, JHIII injections sustain males' aggressive posture during intra-sexual
323 fights (Kou et al., 2008). Here, we highlight that JH treatment upregulated aggressive behaviors
324 associated with reproduction in *E. vuilleti* females, just as testosterone does in vertebrates
325 (Wingfield, 1984). This is an important result because vertebrate and insect hormones have different
326 evolutionary histories and possess different physiological backgrounds, suggesting an evolutionary
327 convergence of endocrine effects across vertebrates and insects (Tibbetts and Crocker, 2014).

328 4.3. Evolution of JH functions across hymenopterans with different degrees of sociality

329 JH has been observed to play two important roles in life history regulation in hymenopteran
330 societies: gonadotropin and behavioral pacemaker (Robinson and Vargo, 1997). Because regulation
331 of reproduction is generally the main role of JH in adult insects (Nijhout, 1994), it is assumed that
332 this was an ancestral function of this hormone in social hymenoptera. So, how the relationship
333 between JH regulation of reproduction and JH regulation of aggressiveness has evolved across
334 hymenopteran taxa with different degree of sociality remains an open question. Currently, two
335 hypotheses have been advanced: i) the 'novel- or single-function hypothesis' (Robinson et al., 1992,
336 Robinson and Vargo, 1997; Agraharia and Gadagkar, 2003) proposes that the role of JH has changed
337 from an exclusively reproductive function in primitively eusocial species (those lacking
338 morphologically distinct queen and worker castes), to an exclusively behavioral function in highly
339 eusocial societies (those containing morphologically distinct castes), whereas ii) the 'split-function
340 hypothesis' (West-Eberhard, 1996) proposes that JH affects regulation of both reproduction and
341 behavior in ancestral non-social species, but that these different effects of JH were then divided
342 between queens and workers in more social species (i.e. regulation of reproductive maturation in
343 egg-laying queens and behavioral pacemaker in workers). Previous studies have lent some support to
344 both hypotheses (Agraharia and Gadagkar, 2003; Giray et al., 2005). To the extent that JH modulates

345 ovarian development and aggressiveness in *E. vuilleti*, a strict non-social hymenopteran, our work
346 supports the split-function hypothesis, but further investigations in non-social hymenopteran species
347 will be essential for comparative studies on the evolution of JH function.

348

349 **5. Conclusion**

350 By testing the role of JH on both reproductive status and aggressiveness in female *E. vuilleti*,
351 we have shown for the first time in a non-social hymenopteran parasitoid that JH has a gonadotropic
352 effect and promotes aggressive behaviors during contests over hosts. Because JH upregulates
353 aggressive behaviors associated with reproduction, our data support the possibility that JH in insects
354 and testosterone in vertebrates have parallel functions, suggesting evolutionary convergence of
355 endocrine systems.

356 **Funding**

357 This work was supported by the French Ministry of Research (2016-Bourse Ministérielle-44).

358

359 **Acknowledgement**

360 We are grateful to Charlotte Lécureuil and Joël Meunier for their advices and Fabrice Vannier for his
361 technical assistance. We thank Dr. Mark Sheridan and 2 anonymous reviewers for helpful comments
362 on the manuscript.

363

364 **References**

- 365 Adkins-Regan, E., 1999. Testosterone increases singing and aggression but not male-typical sexual partner preference in
366 early estrogen treated female zebra finches. *Horm. Behav.* 35, 63–70. <https://doi.org/10.1006/hbeh.1998.1497>
- 367 Adkins-Regan, E., 2005. Hormones and Animal Social Behavior, *The Journal of Nervous and Mental Disease*.
- 368 Agrahari, M., Gadagkar, R., 2003. Juvenile hormone accelerates ovarian development and does not affect age polyethism

in the primitively eusocial wasp, *Ropalidia marginata*. J. Insect Physiol. 49, 217–222.
[https://doi.org/10.1016/S0022-1910\(02\)00268-8](https://doi.org/10.1016/S0022-1910(02)00268-8)

Amsalem, E., Teal, P., Grozinger, C.M., Hefetz, A., 2014. Precocene-I inhibits juvenile hormone biosynthesis, ovarian activation, aggression and alters sterility signal production in bumble bee (*Bombus terrestris*) workers. J. Exp. Biol. 217, 3178–3185. <https://doi.org/10.1242/jeb.107250>

Arnott, G., Elwood, R.W., 2008. Information gathering and decision making about resource value in animal contests. Anim. Behav. <https://doi.org/10.1016/j.anbehav.2008.04.019>

Barth, R.H., Lester, L.J., Sroka, P., Kessler, T., Hearn, R., 1975. Juvenile hormone promotes dominance behavior and ovarian development in social wasps (*Polistes annularis*). Experientia 31, 691–692.
<https://doi.org/10.1007/BF01944632>

Barton, R.A., 1993. Sociospatial mechanisms of feeding competition in female olive baboons, *Papio anubis*. Anim. Behav. 46, 791–802.

Bell, W.J., 1973. Factors controlling initiation of vitellogenesis in a primitively social bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae). Insectes Soc. 20, 253–260. <https://doi.org/10.1007/BF02223194>

Bloch, G., Borst, D.W., Huang, Z., Robinson, G.E., Cnaani, J., Hefetz, A., 2000. Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. J. Insect Physiol. 46, 47–57.

Breed, M.D., Bell, W.J., 1983. Hormonal influences on invertebrate aggressive behavior, in: Svare, B.B. (Ed.), Hormones and Aggressive Behavior. Plenum Press, New York, NY, pp. 577–590.

Brent, C., Peeters, C., Dietmann, V., Crewe, R., Vargo, E., 2006. Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 192, 315–320. <https://doi.org/10.1007/s00359-005-0065-6>

Briffa, M., Hardy, I.C.W., Gammell, M.P., Jennings, D.J., Clarke, D.D., Goubault, M., 2013. Analysis of animal contest data, in: Hardy, I.C.W., Briffa, M. (Eds.), Animal Contests. Cambridge University Press, pp. 47–85.

Brown, W.D., Chimenti, A.J., Siebert, J.R., 2007. The payoff of fighting in house crickets: Motivational asymmetry increases male aggression and mating success. Ethology 113, 457–465. <https://doi.org/10.1111/j.1439-0310.2007.01357.x>

Brown, W.D., Smith, A.T., Moskalik, B., Gabriel, J., 2006. Aggressive contests in house crickets: size, motivation and the information content of aggressive songs. Anim. Behav. 72, 225–233.
<https://doi.org/10.1016/j.anbehav.2006.01.012>

399 Buck, C.L., Barnes, B.M., 2003. Androgen in free-living arctic ground squirrels: Seasonal changes and influence of
400 staged male-male aggressive encounters. *Horm. Behav.* 43, 318–326. [https://doi.org/10.1016/S0018-](https://doi.org/10.1016/S0018-506X(02)00050-8)
401 506X(02)00050-8

402 Chase, I.D., 1974. Models of hierarchy formation in animals societies. *Behav. Sci.* 19, 374–382.

403 Collias, N.E., 1944. Aggressive behavior among vertebrate animals. *Physiol. Zool.* 17, 83–123.
404 <https://doi.org/10.1086/physzool.17.1.30151832>

405 Damiens, D., Imbert, E., Bressac, C., Thibeaudeau, C., Chevrier, C., 2001. Egg-laying, pre-imaginal growth dynamics,
406 and mortality in *Eupelmus orientalis* and *Dinarmus basalis*, two solitary ectoparasitoids of *Callosobruchus*
407 *maculatus*. *Entomol. Exp. Appl.* 99, 97–105. <https://doi.org/10.1023/A:1018954320519>

408 Díaz-Uriarte, R., 1999. Anti-predator behaviour changes following an aggressive encounter in the lizard *Tropidurus*
409 *hispidus*. *Proc. R. Soc. B Biol. Sci.* 266, 2457–2464. <https://doi.org/10.1098/rspb.1999.0946>

410 Dissanayake, A., Galloway, T.S., Jones, M.B., 2009. Physiological condition and intraspecific agonistic behaviour in
411 *Carcinus maenas* (Crustacea: Decapoda). *J. Exp. Mar. Bio. Ecol.* 375, 57–63.
412 <https://doi.org/10.1016/j.jembe.2009.05.007>

413 Emlen, D.J., Nijhout, H.F., 2000. The Development and Evolution of Exaggerated Morphologies in Insects. *Annu. Rev.*
414 *Entomol.* 45, 661–708. <https://doi.org/10.1146/annurev.ecolsys.36.102003.152640>

415 Enquist, M., Leimar, O., 1987. Evolution of fighting behaviour: The effect of variation in resource value. *J. Theor. Biol.*
416 127, 187–205. [https://doi.org/10.1016/S0022-5193\(87\)80130-3](https://doi.org/10.1016/S0022-5193(87)80130-3)

417 Festa-Bianchet, M., Apollonio, M., Mari, F., Rasola, G., 1990. Aggression among Lekking Male Fallow Deer (*Dama*
418 *dama*): Territory Effects and Relationship with Copulatory Success. *Ethology.* 85, 236–246.

419 Field, S.A., Calbert, G., 1999. Don't count your eggs before they're parasitized: contest resolution and the trade-offs
420 during patch defense in a parasitoid wasp. *Behav. Ecol.* 10, 122–127. <https://doi.org/10.1093/beheco/10.2.122>

421 Gauthier, N., Monge, J.P., 1999. Could the egg itself be the source of the oviposition deterrent marker in the
422 ectoparasitoid *Dinarmus basalis*? *J. Insect Physiol.* 45, 393–400. [https://doi.org/10.1016/S0022-1910\(98\)00138-3](https://doi.org/10.1016/S0022-1910(98)00138-3)

423 Giray, T., Giovanetti, M., West-Eberhard, M.J., 2005. Juvenile hormone, reproduction, and worker behavior in the
424 neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci.* 102, 3330–3335.
425 <https://doi.org/10.1073/pnas.0409560102>

426 Goubault, M., Cortesero, A. M., Poinso, D., Wajnberg, E., Boivin, G., 2007. Does host value influence female
427 aggressiveness, contest outcome and fitness gain in parasitoids? *Ethology*, 113(4), 334–343.
428 <https://doi.org/10.1111/j.1439-0310.2006.01325.x>

429 Hammerstein, P., 1981. The role of asymmetries in animal contests. *Anim. Behav.* 29, 193–205.
 430 [https://doi.org/10.1016/S0003-3472\(81\)80166-2](https://doi.org/10.1016/S0003-3472(81)80166-2)

431 Hardy, I.C.W., Briffa, M., 2013. *Animal contests*. Cambridge University Press.

432 Hartfelder, K., 2000. Insect juvenile hormone: from “status quo” to high society. *Brazilian J. Med. Biol. Res.* 33, 157–77.
 433 <https://doi.org/10.1590/S0100-879X2000000200003>

434 Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: Meta-analyses of the
 435 challenge hypothesis. *Anim. Behav.* 71, 265–277. <https://doi.org/10.1016/j.anbehav.2005.04.014>

436 Hirschenhauser, K., Winkler, H., Oliveira, R.F., 2003. Comparative analysis of male androgen responsiveness to social
 437 environment in birds: The effects of mating system and paternal incubation. *Horm. Behav.* 43(4), 508–519.
 438 [https://doi.org/10.1016/S0018-506X\(03\)00027-8](https://doi.org/10.1016/S0018-506X(03)00027-8)

439 Hughes, J. P., Harvey, I. F., Hubbard, S. F., 1994. Host-searching behavior of *Venturia canescens* (Grav.) (Hymenoptera:
 440 Ichneumonidae): Interference-the effect of mature egg load and prior behavior. *J. Insect Behav.* 7(4), 433–454.
 441 <https://doi.org/10.1007/BF02025442>

442 Innocent, T.M., West, S.A., Sanderson, J.L., Hyrkkänen, N., Reece, S.E., 2011. Lethal combat over limited resources:
 443 Testing the importance of competitors and kin. *Behav. Ecol.* 22, 923–931. <https://doi.org/10.1093/beheco/arq209>

444 Jakobsson, S., Brick, O., Kullberg, C., 1995. Escalated fighting behaviour incurs increased predation risk. *Anim. Behav.*
 445 49, 235–239. [https://doi.org/10.1016/0003-3472\(95\)80172-3](https://doi.org/10.1016/0003-3472(95)80172-3)

446 Jaloux, B., Sanon, A., Huignard, J., Monge, J.P., 2004. Interspecific relationships between the solitary ectoparasitoid,
 447 *Eupelmus vuilleti* (Crw.) (Eupelmidae), and its sympatric species, *Dinarmus basalis* (Rond.) (Pteromalidae), in the
 448 presence of their host, *Callosobruchus maculatus* pic (Coleoptera Bruchidae). *J. Insect Behav.* 17, 793–808.
 449 <https://doi.org/10.1023/B:JOIR.0000048989.36181.4d>

450 Kelstrup, H.C., Hartfelder, K., Esterhuizen, N., Wossler, T.C., 2017. Juvenile hormone titers, ovarian status and
 451 epicuticular hydrocarbons in gynes and workers of the paper wasp *Belonogaster longitarsus*. *J. Insect Physiol.* 98,
 452 83–92. <https://doi.org/10.1016/j.jinsphys.2016.11.014>

453 Kelstrup, H.C., Hartfelder, K., Nascimento, F.S., Riddiford, L.M., 2014a. Reproductive status, endocrine physiology and
 454 chemical signaling in the Neotropical, swarm-founding eusocial wasp *Polybia micans*. *J. Exp. Biol.* 217, 2399–
 455 2410. <https://doi.org/10.1242/jeb.096750>

456 Kelstrup, H.C., Hartfelder, K., Nascimento, F.S., Riddiford, L.M., 2014b. The role of juvenile hormone in dominance
 457 behavior, reproduction and cuticular pheromone signaling in the caste-flexible epiponine wasp, *Synoeca surinama*.
 458 *Front. Zool.* 11, 1–19. <https://doi.org/10.1186/s12983-014-0078-5>

459 Kelstrup, H.C., Hartfelder, K., Wossler, T.C., 2015. *Polistes smithii* vs. *Polistes dominula*: the contrasting endocrinology
 460 and epicuticular signaling of sympatric paper wasps in the field. *Behav. Ecol. Sociobiol.* 69, 2043–2058.
 461 <https://doi.org/10.1007/s00265-015-2015-9>
 462 Klukowski, M., Nelson, C.E., 1998. The challenge hypothesis and seasonal changes in aggression and steroids in male
 463 northern fence lizards (*Sceloporus undulatus hyacinthinus*). *Horm. Behav.* 33, 197–204.
 464 <https://doi.org/10.1006/hbeh.1998.1449>
 465 Kou, R., Chou, S.Y., Huang, Z.Y., Yang, R.L., 2008. Juvenile hormone levels are increased in winners of cockroach
 466 fights. *Horm. Behav.* 54, 521–527. <https://doi.org/10.1016/j.yhbeh.2008.05.011>
 467 Lynch, J.W., Ziegler, T.E., Strier, K.B., 2002. Individual and seasonal variation in fecal testosterone and cortisol levels of
 468 wild male tufted capuchin monkeys, *Cebus apella nigratus*. *Horm. Behav.* 41, 275–287.
 469 <https://doi.org/10.1006/hbeh.2002.1772>
 470 Mathiron, A.G.E., Pottier, P., Goubault, M., 2018. Let the most motivated win : resource value components affect contest
 471 outcome in a parasitoid wasp. *Behav. Ecol.* 00, 1–8. <https://doi.org/10.1093/beheco/ary084>
 472 Maynard-Smith, J., Parker, G.A., 1976. The logic of asymmetric animal contests. *Anim. Behav.* 24, 159–175.
 473 Maynard-Smith, J., Price, G. R., 1973. The logic of animal conflict. *Nature*, 246, 15–18.
 474 <https://doi.org/10.1038/246015a0>
 475 Mohamad, R., Monge, J.P., Goubault, M., 2013. Do resource value and ownership status affect intensity and resolution
 476 of contests in a parasitoid wasp? *Entomol. Exp. Appl.* 147, 99–109. <https://doi.org/10.1111/eea.12049>
 477 Mohamad, R., Monge, J.P., Goubault, M., 2012. Wait or fight? Ownership asymmetry affects contest behaviors in a
 478 parasitoid wasp. *Behav. Ecol.* 23, 1330–1337. <https://doi.org/10.1093/beheco/ars125>
 479 Mohamad, R., Monge, J.P., Goubault, M., 2010. Can subjective resource value affect aggressiveness and contest
 480 outcome in parasitoid wasps? *Anim. Behav.* 80, 629–636. <https://doi.org/10.1016/j.anbehav.2010.06.022>
 481 Moore, M.C., 1986. Elevated testosterone levels during nonbreeding-season territoriality in a fall-breeding lizard,
 482 *Sceloporus jarrovi*. *J. Comp. Physiol. A* 158, 159–163. <https://doi.org/10.1007/BF01338559>
 483 Muller, M.N., Wrangham, R.W., 2004. Dominance, aggression and testosterone in wild chimpanzees: A test of the
 484 “challenge hypothesis.” *Anim. Behav.* 67, 113–123. <https://doi.org/10.1016/j.anbehav.2003.03.013>
 485 Neat, F., Huntingford, F., Beveridge, M., 1998. Fighting and assessment in male cichlid fish: the effects of asymmetries
 486 in gonadal state and body size. *Anim. Behav.* 55, 883–91. <https://doi.org/10.1006/anbe.1997.0669>
 487 Nijhout, H.F., 1994. *Insect Hormones*. Princeton University Press, Princeton.
 488 Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canario, A.V.M., 2002. Social modulation of androgen levels in male

489 teleost fish. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* 132, 203–215. <https://doi.org/10.1016/S1096->
490 4959(01)00523-1

491 Palombit, R.A., 1993. Lethal territorial aggression in a white-handed gibbon. *Am. J. Primatol.* 31, 311–318.
492 <https://doi.org/10.1002/ajp.1350310407>

493 Pearce, A.N., Huang, Z.Y., Breed, M.D., 2001. Juvenile hormone and aggression in honey bees. *J. Insect Physiol.* 47,
494 1243–1247. [https://doi.org/10.1016/S0022-1910\(01\)00109-3](https://doi.org/10.1016/S0022-1910(01)00109-3)

495 Petersen, G., Hardy, I.C.W., 1996. The importance of being larger: parasitoid intruder – owner contests and their
496 implications for clutch size. *Anim. Behav.* 51, 1363–1373. <https://doi.org/10.1006/anbe.1996.0139>

497 Ramaseshadri, P., Farkaš, R., Palli, S.R., 2012. Recent Progress in Juvenile Hormone Analogs (JHA) Research, 1st ed,
498 Advances in Insect Physiology. Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-391500-9.00005-X>

499 Robinson, G.E., 1992. Regulation of Division of Labor in Insect Societies. *Annu. Rev. Entomol.* 37, 637–665.
500 <https://doi.org/10.1146/annurev.en.37.010192.003225>

501 Robinson, G.E., Vargo, E.L., 1997. Juvenile hormone in adult eusocial hymenoptera: Gonadotropin and behavioral
502 pacemaker. *Arch. Insect Biochem. Physiol.* 35, 559–583. [https://doi.org/10.1002/\(SICI\)1520-](https://doi.org/10.1002/(SICI)1520-)
503 6327(1997)35:4<559::AID-ARCH13>3.0.CO;2-9

504 Röseler, P.F., 1991. Reproductive competition during colony establishment, in: Ross, K.G., Matthews, R.W. (Eds.), *The*
505 *Social Biology of Wasps*. Cornell University Press., Ithaca, NY, pp. 309–335.

506 Röseler, P.F., Röseler, I., Strambi, A., Augier, R., 1984. Influence of insect hormones on the establishment of dominance
507 hierarchies among foundresses of the paper wasp, *Polistes gallicus*. *Behav. Ecol. Sociobiol.* 15, 133–142.
508 <https://doi.org/10.1007/BF00299381>

509 Röseler, P.F., Röseler, I., Strambi, A., 1980. The Activity of Corpora Allata in Dominant and Subordinated Females of
510 the Wasp *Polistes Gallicus*. *Insectes Soc.* 27, 97–107.

511 Rowland, W.J., 1989. The effects of body size, aggression and nuptial coloration on competition for territories in male
512 threespine sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* 37, 282–289. <https://doi.org/10.1016/0003->
513 3472(89)90117-6

514 Salvador, A., Veiga, J.P., Martin, J., Lopez, P., Abelenda, M., Puerta, M., 1996. The cost of producing a sexual signal:
515 Testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behav. Ecol.* 7, 145–150.
516 <https://doi.org/10.1093/beheco/7.2.145>

517 Sasagawa, H., Sasaki, M., Okada, I., 1989. Hormonal Control of the Division of Labor in Adult Honeybees (*Apis*
 518 *mellifera* L.) I. Effect of Methoprene on Corpora Allata and Hypopharyngeal Gland, and Its α -Glucosidase
 519 Activity. Japanese Soc. Appl. Entomol. Zool. 24, 66–77.

520 Schwagmeyer, P.L., Woontner, S.J., 1985. Mating Competition in an Asocial Ground-Squirrel, *Spermophilus*
 521 *tridecemlineatus*. Behav. Ecol. Sociobiol. 17, 291–296. <https://doi.org/10.1007/BF00300149>

522 Scott, M.P., 2006. The role of juvenile hormone in competition and cooperation by burying beetles. J. Insect Physiol. 52,
 523 1005–1011. <https://doi.org/10.1016/j.jinsphys.2006.04.006>

524 Simmons, L., 1986. Intermale Competition and Mating Success in the Field Cricket, *Gryllus bimaculatus* (Degeer).
 525 Anim. Behav. 34, 567–579. [https://doi.org/10.1016/S0003-3472\(86\)80126-9](https://doi.org/10.1016/S0003-3472(86)80126-9)

526 Sláma, K., 1971. Insect juvenile hormone analogues. Annu. Rev. Biochem. 40, 1079–102.
 527 <https://doi.org/10.1146/annurev.bi.40.070171.005243>

528 Smith, A.R., Kapheim, K.M., Pérez-Ortega, B., Brent, C.S. & Wcislo, W.T. 2013. Juvenile hormone levels reflect social
 529 opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera : Halictidae). Horm. Behav.
 530 63, 1–4.

531 Smith, J.M., Price, G.R., 1973. The logic of animal conflict. Nature 246, 15–18. <https://doi.org/10.1038/246015a0>

532 Stokkebo, S., Hardy, I.C.W., 2000. The importance of being gravid: egg load and contest outcome in a parasitoid wasp.
 533 Anim. Behav. 59, 1111–1118. <https://doi.org/10.1006/anbe.2000.1407>

534 Terrasse, P.C., Rojas-Rousse, D., 1986. Distribution de la ponte et évitement du superparasitisme chez l'hyménoptère
 535 solitaire *Bruchocida vuilleti* Cw. (Hym., Eupelmidae), parasite des stades larvaires de son hôte, *Callosobruchus*
 536 *maculatus* F. (Col., Bruchidae). J. Appl. Entomol. 101, 243–256. <https://doi.org/10.1111/j.1439-0418.1986.tb00855.x>

537

538 Tibbetts, E.A., Crocker, K.C., 2014. The challenge hypothesis across taxa: Social modulation of hormone titres in
 539 vertebrates and insects. Anim. Behav. 92, 281–290. <https://doi.org/10.1016/j.anbehav.2014.02.015>

540 Tibbetts, E.A., Huang, Z.Y., 2010. The Challenge Hypothesis in an Insect: Juvenile Hormone Increases during
 541 Reproductive Conflict following Queen Loss in *Polistes* Wasps. Am. Nat. 176, 123–130.
 542 <https://doi.org/10.1086/653664>

543 Tibbetts, E.A., Izzo, A.S., 2009. Hormones and Behavior Endocrine mediated phenotypic plasticity : Condition-
 544 dependent effects of juvenile hormone on dominance and fertility of wasp queens. Horm. Behav. 56, 527–531.
 545 <https://doi.org/10.1016/j.yhbeh.2009.09.003>

546 Tibbetts, E.A., Vernier, C., Jinn, J., 2013. Juvenile hormone influences precontest assessment behaviour in *Polistes*

547 *dominulus* paper wasps. Anim. Behav. 85, 1177–1181. <https://doi.org/10.1016/j.anbehav.2013.03.003>

548 Tobe, S.S., Stay, B., 1985. Structure and Regulation of the Corpus Allatum. Adv. In Insect Phys. 18, 305–432.

549 [https://doi.org/10.1016/S0065-2806\(08\)60043-0](https://doi.org/10.1016/S0065-2806(08)60043-0)

550 Vogel, E.R., 2005. Rank differences in energy intake rates in white-faced capuchin monkeys, *Cebus capucinus*: The

551 effects of contest competition. Behav. Ecol. Sociobiol. 58, 333–344. <https://doi.org/10.1007/s00265-005-0960-4>

552 West-Eberhard, M., 1996. Wasp societies as microcosms for the study of development and evolution, in: Turillazzi, S.,

553 West-Eberhard, M. (Eds.), Natural History and Evolution of Paper Wasps. Oxford University Press, Oxford, pp.

554 290–31.

555 West-Eberhard, M.J., 1979. Sexual Selection, Social Competition, and Evolution. Proc. Amercian Philos. Soc. 123, 222–

556 234.

557 Wheeler, D., 1996. The Role of Nourishment in Oogenesis. Annu. Rev. Entomol. 41, 407–431.

558 <https://doi.org/10.1146/annurev.ento.41.1.407>

559 Wiesel, G., Tappermann, S., Dorn, A., 1996. Effects of Juvenile Hormone and Juvenile Hormone Analogues on the

560 Phase Behaviour of *Schistocerca gregaria* and *Locusta migratoria*. J. Insect Physiol. 42, 385–395.

561 Wilson, K., Hardy, I.C.W., 2002. Statistical analysis of sex ratios: an introduction, in: Hardy, I.C.W. (Ed.), Sex Ratios:

562 Concepts and Research Methods. Part 2. Statistical Analysis of Sex Ratio Data. Cambridge University Press, New

563 York, pp. 46–92.

564 Wingfield, J.C., 1984. Androgens and Mating Systems: Testosterone-Induced Polygyny in Normally Monogamous Birds.

565 Auk 101, 665–671. <https://doi.org/10.2307/4086893>

566 Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The “Challenge Hypothesis”: Theoretical Implications for

567 Patterns of Testosterone Secretion, Mating Systems, and Breeding Strategies. Am. Nat. 136, 829–846.

568 <https://doi.org/10.1086/285134>

569 Zera, A.J., 2007. Endocrine analysis in evolutionary-developmental studies of insect polymorphism: Hormone

570 manipulation versus direct measurement of hormonal regulators. Evol. Dev. 9, 499–513.

571 <https://doi.org/10.1111/j.1525-142X.2007.00181.x>