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2 Caste-fate determination primarily occurs after adult emergence in a primitively

3 eusocial paper wasp: Significance of the photoperiod during the adult stage

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Abstract Independent-founding paper wasps constitute a major group of primitively eusocial insects, and when caste-fate determination occurs in temperate species of these wasps, particularly regarding whether it occurs before or after emergence, remains unclear. No critical morphological differences occur between potential queens of the next generation (often called gynes) and workers in primitively eusocial insects. The gynes of temperate species are characterized by diapausing, and the nutrients available during the larval stage have often been believed to determine caste fate. Short days usually induce diapause in temperate nonsocial insects, although few investigations of the effects of day length on caste-fate determination in paper wasps have been conducted. By exposing individuals to different combinations of short and long days during the immature and adult stages, we show for the first time that short days during the adult stage (but not during the immature stage) facilitated caste-fate determination toward gynes in a paper wasp. Moreover, the decision to diapause partly depended on changes in the photoperiod during the pupal and adult stages. The size of the adult also affected caste-fate determination, with diapause more likely to occur in large adults, but this size effect did not occur when individuals were exposed to many short days during the pupal stage. In addition, all adults except for a small proportion of smaller individuals prepared for diapause under short days.

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34 These findings suggest that the photoperiod is a higher priority cue than adult size.

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36 **Keywords** diapause · egg maturation · lipid stores · Polistinae · social insects · Vespidae

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38 **Introduction**

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40 The origin of eusociality, that is, the origin of workers, is an intriguing topic for anyone
41 interested in biology or sociology. The related topic of how caste-fate determination
42 occurs has attracted those interested in research on social insects because our
43 understanding of the origin of eusociality will improve (Oster and Wilson 1978; Hunt
44 1991; Smith et al. 2008). Primitively eusocial insects are the most suitable animals
45 for these investigations because no critical morphological differences occur between the
46 queen and workers (Dapporto et al. 2011; Petrocelli and Turillazzi 2013), which
47 appears to represent an early stage of eusociality (Hunt 2012).

48 Considerable controversy occurs among researchers about when caste-fate
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50 determination occurs in temperate species of primitively eusocial paper wasps,
51 particularly regarding whether the determination occurs before or after emergence,
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57 corresponding to the preimaginal and imaginal caste-fate determination hypotheses,

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3 52 respectively (O'Donnell 1998). The hypothesis of preimaginal caste-fate determination
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6 53 assumes that the caste fate is determined during the immature stage through
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9 54 qualitative and/or quantitative differences in diet and/or mechanical stimuli (O'Donnell
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12 55 1998; Hunt 2006; Hunt et al. 2007; Jeanne and Suryanarayanan 2011). Members of
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15 56 the first brood of primitively eusocial paper wasps, which are nursed by the foundress
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18 57 or foundresses, are often considered destined to be workers at emergence (Hunt 2006;
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21 58 Hunt et al. 2007) because they are small and light (Reeve 1991) and have low lipid
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24 59 stores (Judd et al. 2010). This type of caste-fate determination is typically found in
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27 60 advanced eusocial bees (see for review Hartfelder and Emlen 2012): advanced eusocial
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30 61 insects are characterized primarily by clear morphological differences between the
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33 62 queen and workers (Wilson 1971; Michener 1974; Bourke 1999; Jeanne 2003).
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38 63 The imaginal caste-fate determination hypothesis assumes that the caste fate is
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41 64 determined after emergence based on several cues (Solís and Strassmann 1990; Reeve
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44 65 et al. 1998; Tibbetts 2007). However, one supporter of this hypothesis, Tibbetts et al.
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47 66 (2011), suggested that body weight at emergence influences fertility. The caste fate of
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50 67 emerging wasps reportedly changes with colony conditions, such as the presence of a
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53 68 brood (Solís and Strassmann 1990), colony size, and the presence of the foundress
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queen (Tibbetts 2007). These reports support the imaginal caste-fate determination hypothesis.

The potential queens of the next generation (often called gynes) are characterized by diapausing in temperate paper wasps; therefore, the above question on caste-fate determination can be addressed based on when the female decides to enter diapause. The gynes store lipids for diapausing and refrain from developing ovaries, whereas nondiapausing individuals develop ovaries and refrain from storing lipids, that is, they have more and larger oocytes and smaller lipid stores than those of the gynes (Eickwort 1969; Haggard and Gamboa 1980; Strassmann et al. 1984; Toth et al. 2009). These differences are assumed to be more distinct when sufficient food is available and social interactions are not present. The gynes usually have no mature eggs, although an exceptional species has recently been reported (Kelstrup et al. 2017).

Day length has been considered to play a critical role in the induction of diapause among temperate nonsocial insects (Tauber et al. 1986; Danks 1987; Saunders 2002), with short days usually inducing diapause. However, we know of only one study that has investigated the effects of day length on caste-fate determination. Bohm (1972) found that emerging adults placed under conditions of a 16-h light/8-h dark photoperiod (LD 16:8) and 26°C were more likely to develop ovaries

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3 87 than those placed under conditions of LD 14:10 and 22°C. This phenomenon was
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6 88 found only among adults emerging from nests collected in June, whereas most of the
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9 89 adults emerging from nests collected in July and August entered diapause. From
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12 90 these observations, Bohm inferred that immatures also sensed cues related to
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15 91 diapausing. Although Bohm's observations suggest that temperature and/or the
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18 92 photoperiod could be important factors for inducing diapause, many researchers
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22 93 (including Hunt et al. 2007) disagree with the importance of these factors, most likely
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25 94 because of the occurrence of early gynes (Reeve et al. 1998) and late workers (Dapporto
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28 95 et al. 2005). These discrepancies indicate that some questions remained to be
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31 96 resolved, such as which developmental stage is sensitive to day length.
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35 97 When insects enter diapause as adults, sensitivity to day length during the
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38 98 larval, pupal, or adult stage is assumed the cause (Danks 1987). If the day length
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41 99 during the adult stage is critical, then the caste fate of newly emerged females is not
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44 100 predetermined, which would support the imaginal caste-fate determination hypothesis
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47 101 and underscore that flexibility occurs in the caste fate in primitively eusocial wasps.
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51 102 In this study, we used the paper wasp *Polistes jokahamae* (nee *P. jadvigae*)
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54 103 Radoszkowski (Hymenoptera: Vespidae; Polistinae) to explore the effects of day length
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57 104 on caste-fate determination; in particular, to elucidate which developmental stage is
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105 sensitive to day length. The effects of adult size were also analyzed to determine
106 possible influences of development during the immature stage.

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108 **Materials and Methods**

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110 The target insect

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112 *Polistes jokahamae* is a solitary-founding paper wasp that is common in Japan.

113 Overwintered foundresses emerge from diapause in late March and found a nest

114 solitarily from mid-April to early May in Mie and Aichi. Workers emerge from late

115 May to July, followed by the emergence of males and gynes.

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117 Collecting and rearing

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119 Nests with the foundress were collected before worker emergence in early May in Mie

120 and Aichi and reared individually in cardboard boxes 30 cm × 30 cm × 45 cm whose top

121 and three sides were replaced by vinyl sheeting. Fourth- or fifth-instar silk moth

122 larvae, honey, water, and filter paper for nest materials were placed in the rearing box.

Sufficient moth larvae were provided to ensure that they were available to the wasps
ad libitum. Wasps were marked by paint (PAINT MARKER SR; Teranishi Chemical
Industry Company, Osaka, Japan) on their emerging days, and their head width was
measured. In total, 18 rearing boxes containing a nest and foundress were placed in a
room at 25°C and LD 14:10 until the morning of May 15. The LD 14:10 is similar to
the field photoperiod in early May: the day length experienced by immature wasps in
the field was assumed to be from sunrise to sunset plus 0.5 h (not 1 h), based on nests
usually founded in shaded areas such as under eaves.

Treatments

We performed the following experimental procedures to expose immatures to different
photoperiods, with the adults exposed to different photoperiods independently of the
photoperiod exposure of the immatures (Fig. 1). On the morning of May 15, the
rearing boxes containing one colony each were divided into three groups that each
consisted of six boxes, and each group was placed under LD 16:8 (1 h longer than that
of the day length on the summer solstice) for 2, 6, or 12 weeks after which all rearing
boxes were placed under LD 12:12 (similar to the day length in mid-October) (Fig. 1).

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3 141 With this procedure, the individual wasps experienced different photoperiods during
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6 142 the immature stage: some experienced short days (LD 12:12) throughout the immature
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9 143 stage, whereas others experienced long days (LD 16:8) throughout.
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13 144 Every second emerging female was removed from the nest and individually
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16 145 placed into a small, transparent plastic cup (12 cm in diameter \times 6 cm in height)
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19 146 containing one silk moth larva, honey, and water and reared for 2 weeks under LD 16:8
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22 147 or LD 12:12. The survival rate of females for the 2-week rearing period was 96.4% (n
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25 148 = 222). Half of the emerging female wasps were left on the nest to maintain the
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28 149 colony activity. The different combinations of short and long days during the
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31 150 immature and adult stages were assumed to inform the wasps about what part of the
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34 151 year they were in, based on the assumption that wasps used the photoperiod as a cue.
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38 152 After the 2-week rearing period, each female was dissected to determine
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41 153 whether she had mature eggs, and then the dry weight of lipids in the abdomen
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44 154 (strictly speaking, the gaster) was measured using the method described by Tibbetts et
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47 155 al. (2011). An increase in lipid stores indicates that a wasp is preparing for diapause,
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50 156 whereas egg maturation indicates that the wasp will refrain from diapausing (Eickwort
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53 157 1969; Haggard and Gamboa 1980; Strassmann et al. 1984; Toth et al. 2009).
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57 158 Furthermore, female adults collected in mid-November and late December had no
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3 159 mature eggs, but they had many immature eggs of which some were longer than half
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6 160 the length of mature eggs (H. Yoshimura and Y.Y. Yamada, unpublished data).
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9 161 Therefore, the existence of mature eggs was considered to indicate the decision not to
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19 164 Data analyses
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25 166 We performed logistic regression analyses of the effects of the following three factors on
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28 167 egg maturation (proportion of female wasps with mature eggs): (1) photoperiod during
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31 168 the adult stage, (2) photoperiod during the immature (larval or pupal) stage, and (3)
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34 169 adult size (head width). The effects of colonies were also incorporated into a statistical
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37 170 model as a random (stratum) factor. The number of long days (or short days)
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43 172 the photoperiod during the immature stage. The statistical significances of these
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46 173 factors and two-way interactions between them were assessed by calculating exact
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52 175 Cambridge, MA, USA). Because we could not test the significance of some
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55 176 interactions using this software, we performed a log-linear analysis using the lme4
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package in R (version 3.4.1; R Foundation 2017). A logit link function was applied, with the colonies incorporated as random factors, including random slopes against head width and random intercepts. In each analysis, we first fitted potential explanatory variables (photoperiod during the adult stage, photoperiod during the immature stage, and head width) and their two-way interactions. Starting with the interactions, we tested the significance of each term separately, and any term that was not significant was removed from the model. We report *P*-values for individual terms: ones for nonsignificant terms were obtained when removing them, and ones for significant terms were obtained when removing them from the minimally adequate model.

The effects of the above three factors on lipid stores were analyzed using a linear mixed model implemented in NCSS (version 11; NCSS Statistical Software, Kaysville, UT, USA). The presence or absence of mature eggs was added as a fixed factor. The colonies were incorporated as random factors, including random slopes against head width and random intercepts. Log-transformed values of lipid stores were used to assure the normality of random errors.

The effects of treatment and colony phase on egg maturity and lipid stores were also analyzed by incorporating them separately in the model as a fixed factor

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3 195 instead of the photoperiod during the immature stage. The effects of colony phase
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6 196 were analyzed to verify whether wasps emerging late were likely to enter diapause.
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9 197 We divided the emerging wasps into three groups: (1) wasps from the first brood, (2)
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12 198 wasps emerging after the first brood and before the emergence of males, and (3) wasps
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15 199 emerging on the same day as or later than the first-emerging male (often defined as
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18 200 gynes). This analysis revealed that these factors did not exert significant effects;
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22 201 therefore, the results of the analysis are not presented here.
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203 **Results**

204 205 **Egg maturation**

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207 The distribution of the head widths of female adults under long days of the adult stage
208 was almost the same as that under short days (Fig. 2): the head width ranged from
209 3.74 to 4.85 mm for long days and from 3.62 to 4.95 mm for short days, with the
210 frequency peaking between 4.25 and 4.50 mm for both. The adult size and
211 photoperiod during the adult stage significantly affected egg maturation (proportion of
212 female adults with mature eggs; Table 1, Fig. 2). Female adults were more likely to

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213 have mature eggs under long days than under short days; in particular, large adults
214 had no mature eggs under short days.

215 Using the number of long days during the pupal stage as a variable
216 representing the photoperiod during the immature stage revealed an interaction
217 between the adult size and photoperiod during the adult stage. This result indicated
218 that the proportion of adults with mature eggs decreased more rapidly with increasing
219 head widths under short days than under long days. To explore mechanisms
220 underlying the interaction, we performed analyses separately for adults with 0–8 long
221 days during the pupal stage and those with >8 long days during the pupal stage (the
222 former experienced more short days during the pupal stage than did the latter) (Table
223 1, Fig. 3). After the wasps experienced 0–8 long days during the pupal stage, the
224 interaction between the adult size and photoperiod during the adult stage was lost, and
225 the head width of the adult exerted no effect under both short and long days.

226 However, after experiencing >8 long days during the pupal stage, the interaction
227 between the adult size and photoperiod during the adult stage remained, and the head
228 width exerted significant effects. This difference resulted in an interaction between
229 the head width and photoperiod during the pupal stage that was close to statistical
230 significance (Table 1).

Moreover, using the number of long days during the pupal stage for the photoperiod during the immature stage revealed an interaction between the photoperiod during the immature stage and the photoperiod during the adult stage (Table 1). To explore the mechanisms underlying this interaction, we performed analyses separately for adults under short and long days during the adult stage. The photoperiod during the pupal stage had positive effects (approaching statistical significance, $P = 0.096$) on egg maturation among adults under short days (Table 1, Fig. 4), whereas no effect was exerted among adults under long days ($P = 1.000$). This difference in the relationship for adults under short versus long days was considered to generate the above significant interaction between the photoperiod during the pupal stage and the photoperiod during the adult stage.

This interaction suggests that the females responded to changes in day length during the pupal and adult stages; females estimate when the summer solstice has passed by sensing changes in day length during the pupal and adult stages. For example, females exposed to short days during the pupal and adult stages would assume that the solstice passed a long time ago, whereas those exposed to long days during the pupal stage and short days during the adult stage would assume that the solstice only recently passed. As a result, the former would be more likely to refrain

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19 254 whereas that during the immature stage had no effect (Table 2), irrespective of
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25 256 larval or pupal period was targeted (only the results for the statistical analysis of the
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28 257 number of long days during the pupal stage are presented). A significant interaction
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32 258 occurred between the adult size and the presence or absence of mature eggs; therefore,
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35 259 analyses were performed separately for female adults with and without mature eggs
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38 260 (Table 2, Fig. 5). Significant effects of the adult size and photoperiod during the adult
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41 261 stage were revealed among females without mature eggs, whereas no such effects were
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44 262 found among those with mature eggs. This effect of the photoperiod suggests that the
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47 263 females preparing for diapause started to store lipids earlier or stored them more
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57 266 **Discussion**
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268 The effects of photoperiod during the adult stage on egg maturation and lipid stores
269 supported imaginal caste-fate determination, with short days inducing diapause, which
270 facilitated the caste-fate determination toward gynes. The effects of adult size
271 supported preimaginal caste-fate determination. However, because all adults except
272 for some of the smaller ones prepared for diapause under short days, and no effects of
273 the head width on egg maturation were present when individuals were exposed to
274 many short days during the pupal stage, the photoperiod is considered a higher-
275 priority cue than adult size. Notably, many females had matured eggs under long
276 days during the adult stage irrespective of whether they emerged early or late,
277 suggesting that females that are often referred to as gynes are not actually destined to
278 become a specific caste at emergence.

279 Small adults, usually the first brood, were more likely to refrain from entering
280 diapause. The queen may regulate the adult size of the first brood by changing the
281 quality and quantity of the diet and/or producing mechanical stimuli (O'Donnell 1998;
282 Hunt 2006; Hunt et al. 2007; Jeanne and Suryanarayanan 2011). Alternatively, the
283 small adults of the first brood might be only attributable to shorter days, cooler
284 weather, and lack of foragers (only the queen forages) before worker emergence, which

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3 285 result in reduced availability of provisions (Judd et al. 2015). Indeed, the first brood
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6 286 of *P. jokahamae* is small and thin at emergence, resulting in a low weight relative to
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9 287 the body length (H. Yoshimura and Y. Y. Yamada, unpublished data). By contrast,
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15 289 diapause. This finding can be attributed to the fact that large adults likely have
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18 290 higher lipid stores at emergence (H. Yoshimura and Y. Y. Yamada, unpublished data;
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21 291 see Judd et al. 2010) and that diapausing females must have ample lipid stores to
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24 292 successfully overwinter (Toth et al. 2009; Hahn and Denlinger 2011). However, the
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27 293 amount of lipid stores at emergence is much lower than that of the lipid stores required
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30 294 to successfully overwinter: the former is approximately 13 mg, and the latter is
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33 295 approximately 30 mg in *P. jokahamae* gynes (H. Yoshimura and Y. Y. Yamada,
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36 296 unpublished data). Therefore, even gynes must obtain a considerable amount of
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39 297 nutrition after emergence to ensure successful overwintering.
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44 298 Notably, when individuals experienced many short days during the pupal
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47 299 stage, no effects of the head width on egg maturation were present, although the
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50 300 photoperiod during the adult stage had an effect, that is, very few adults matured eggs
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53 301 under short days during the adult stage, whereas many adults did so under long days,
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56 302 irrespective of their head widths. These results suggested that the members of the
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3 303 first brood are likely to mature eggs due to experiencing an increasing day length
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6 304 during the pupal and adult stages, rather than due to a combination of their smallness
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9 305 and long days during the adult stage. The changes in day length employed in the
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12 306 present study differed from those experienced in the field; the day length changed
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18 308 future studies should explore whether the members of the first brood actually use their
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21 309 size as a cue in the field when they decide to mature eggs.
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25 310 An effect of body size on diapause induction is found in the blowfly *Calliphora*
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28 311 *vicina*, with small larvae less likely to enter diapause under diapause-inducing
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31 312 conditions (Saunders 1997). Moreover, a high-quality diet is required to induce
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34 313 diapause in a few insects. Adults of the phytophagous beetle *Epilachna*
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37 314 *vigintioctopunctata* (Kono 1979) and larvae of the bollworm *Pectinophora gossypiella*
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40 315 (Adkisson et al. 1963; Foster and Crowder 1980) enter diapause when they are fed
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43 316 high-quality food under short days but do not when they are fed low-quality food.
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46 317 These phenomena suggest that these insects avoid diapause when the amount of
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49 318 nutrition is insufficient to ensure successful overwintering. A similar phenomenon
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52 319 likely occurred in *P. jokahamae*. However, the effect of body size or nutrition on the
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55 320 decision to diapause is found under diapause-inducing conditions in the above fly and
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3 321 phytophagous insects, whereas in *P. jokahamae*, the effect was present under not only
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6 322 short but also long days, that is, even under conditions that did not induce diapause.
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9 323 The caste fate of emerging adults appears to be determined by several cues
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12 324 that they sense during a certain period after their emergence (Solís and Strassmann
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15 325 1990; Reeve et al. 1998; Tibbetts 2007). These cues include their physiological states
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18 326 (e.g., lipid stores), social interactions with colony members (e.g., dominance hierarchy),
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21 327 colony states (e.g., colony size, the fertility of the queen, and presence or absence of the
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24 328 queen), and physical and biological environmental conditions (e.g., day length and food
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27 329 availability). The wasps appear to sense the day length and changes therein to
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30 330 estimate what part of the year they are in and how many days are left until the
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33 331 beginning of autumn or winter. The idea that caste-fate determination occurs
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36 332 primarily after emergence is supported circumstantially by the nourishment restriction
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39 333 only partially influencing the caste-associated physiology and genes in the paper wasp
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42 334 *Polistes metricus* (Berens et al. 2015; Judd et al. 2015). Only a small proportion of
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45 335 paper wasp colonies manage to develop well and produce many males and gynes in the
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48 336 field (Reeve 1991; Queller 1996; Kozyra and Baraniak 2016), which is due to the effects
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51 337 of unpredictable adverse factors on colonies (Yamane 1996) such as food shortage,
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54 338 attack by predators, parasitoids, and nest-parasitic insects, the loss of the queen, and
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339 harsh weather including strong wind and rain that can destroy the nest. Under such
340 unpredictable field conditions, multiple options at emergence is more adaptive for
341 females (Solís and Strassmann 1990), including entering diapause, staying in the nest,
342 and founding a new nest. Whether the effects of photoperiod on caste fate commonly
343 occur in different paper wasp species is an intriguing question.

344 The above hypothesis strongly supports that at least one mechanism
345 underlying primitive eusociality involves the decision of early emerging females to stay
346 in their natal nests as a helper or worker. This scenario is superficially the same as
347 the diapause ground-plan hypothesis (Hunt 1991, 2006) in terms of emerging adults
348 exhibiting size-related bias in caste-fate determination. However, although the
349 diapause ground-plan hypothesis stresses that the bias is sufficiently strong that
350 preimaginal caste-fate determination occurs, our scenario indicates that the bias is not
351 absolute: the emerging adults have the final say. Moreover, the diapause ground-plan
352 hypothesis assumes that eusociality originates in temperate solitary wasps, whereas
353 our scenario does not. The assumption is now challenged by two facts, as recently
354 suggested by Kelstrup et al. (2017): (1) a recent phylogenetic and biogeographical
355 analysis of *Polistes* paper wasps suggests that the genus originated in a tropical area
356 (Santos et al. 2015), and (2) very few temperate solitary wasps overwinter as adults

(Evan and West-Eberhard 1970). Therefore, after eusociality was established in some tropical wasps, the social wasps likely developed diapause mechanisms, including ones that induce and prepare for diapause, as they expanded their distribution toward temperate areas. Simultaneously, their life history apparently changed, with male production and mating action occurring within a certain period. Exploration of how social wasps developed diapause mechanisms and their attendant changes in life history is of great interest. Species or sibling species with a wide distribution ranging from tropical (or subtropical) to temperate areas are ideal target insects, and comparing characteristics related to diapausing and life history between populations in tropical (or subtropical) and temperate areas will be fruitful.

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Figure captions

Fig. 1 Experimental procedures for determining the effects of photoperiod during the immature and adult stages on diapausing

Fig. 2 Effects of the photoperiod during the adult stage and body size (head width) on egg maturation (proportions of female adults with mature eggs). Data (bars) are the proportions of female adults with mature eggs classified by their head widths. The distribution of head widths is also shown separately for wasps under short and long days (lines). See Table 1 for the results of the statistical analyses

Fig. 3 Effects of the photoperiod during the adult stage and body size (head width) on egg maturation (proportions of female adults with mature eggs) after exposure to different photoperiods (a, 0–8 long days; b, >8 long days) during the pupal stage. Data (bars) are the proportions of female adults with mature eggs classified by their head widths. The distribution of head widths is also shown separately for wasps with 0–8 and >8 long days during the pupal stage (lines). See Table 1 for the results of the statistical analyses

Fig. 4 Effects of the photoperiod (number of long days) during the pupal stage on egg

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497 maturation (proportions of female adults with mature eggs) under different
498 photoperiods (LD 12:12 and LD 16:8) during the adult stage. Data (bars) are
499 proportions of female adults with mature eggs classified by the number of long days
500 during the pupal stage; numbers on the bars are sample sizes. See Table 1 for the
501 results of the statistical analyses
502
Fig. 5 Relationship between lipid stores and body size (head width) under short and
503 long days: a, females without mature eggs; b, females with mature eggs. Lines
504 represent the best-fit linear model in panel a, whereas lines in panel b represent
505 regression models estimated separately for short and long days: no significant
506 relationships were detected (see Table 2 for details)
507

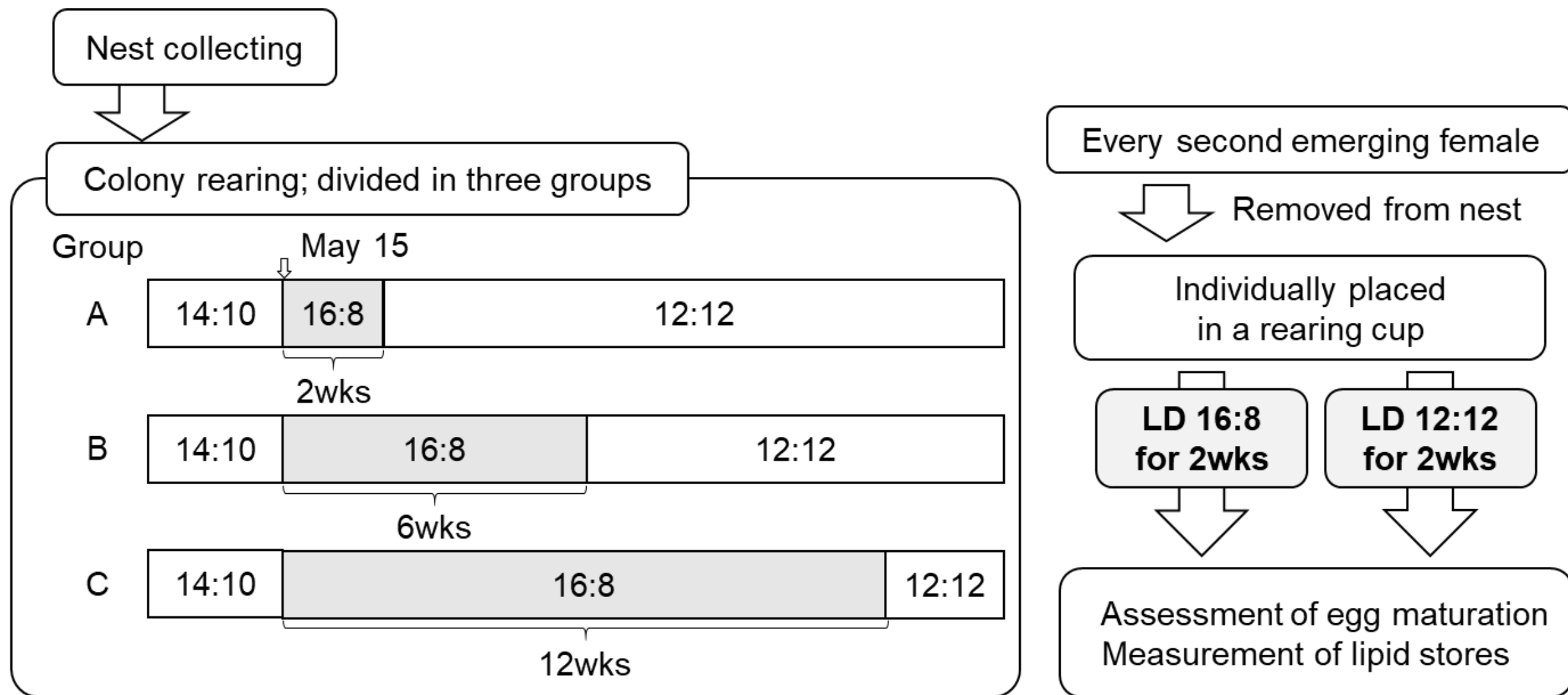
Fig. 1

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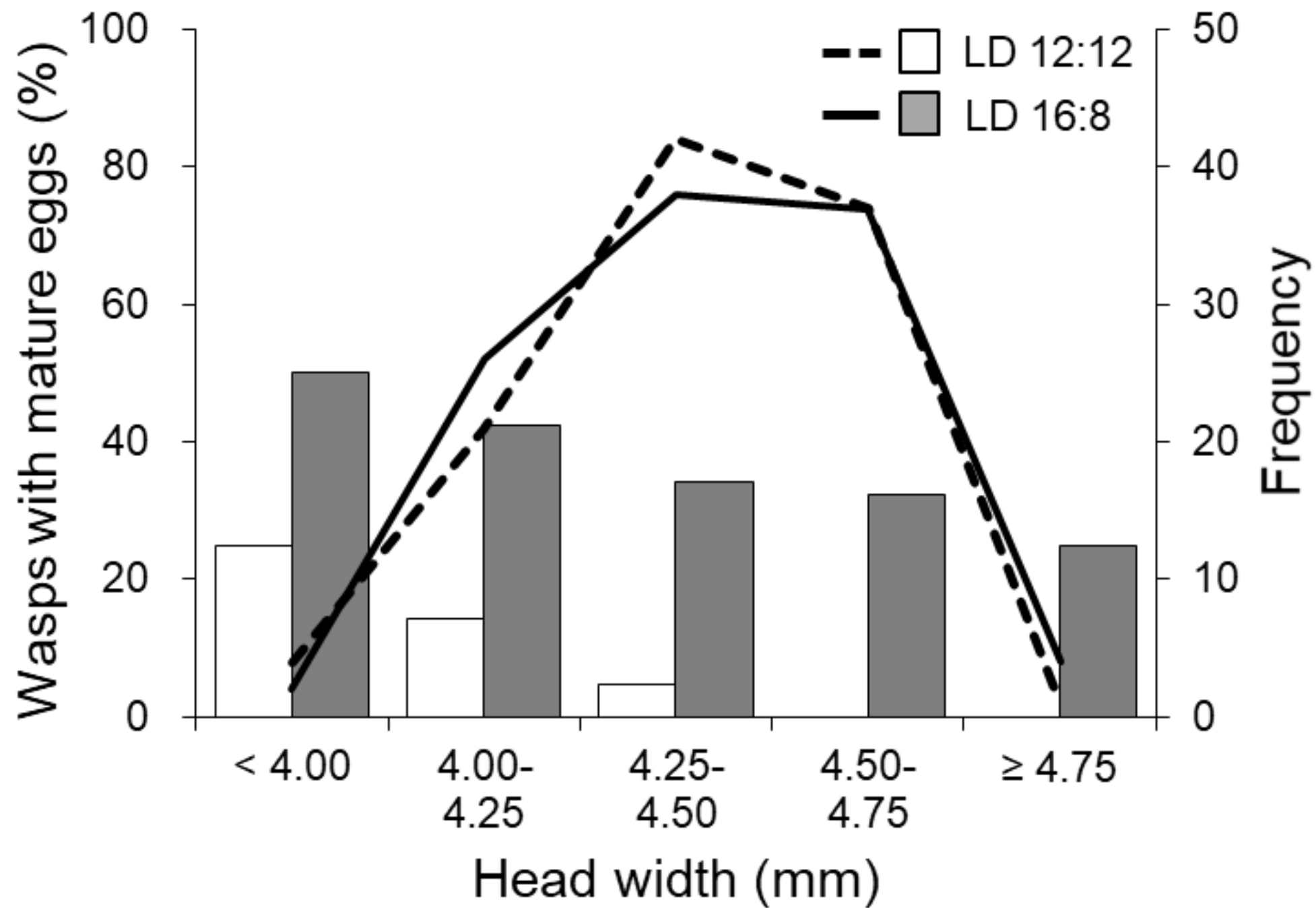


Fig. 3

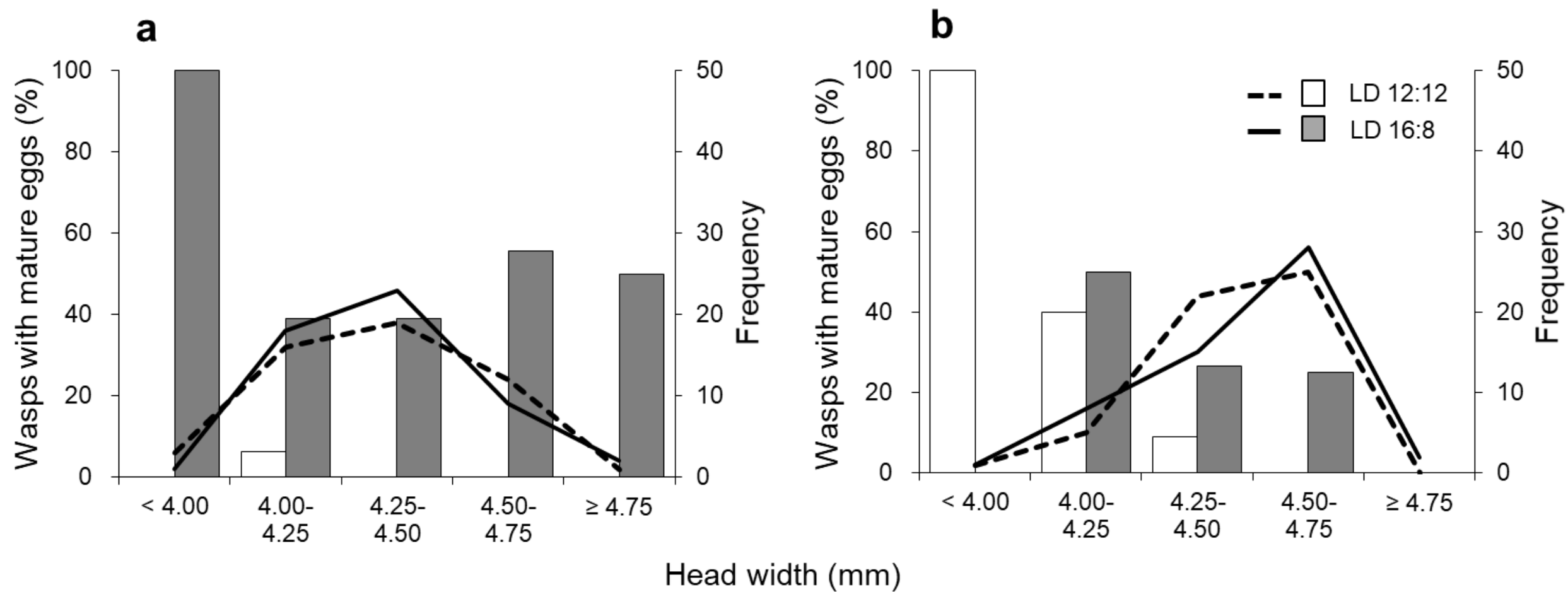


Fig. 4

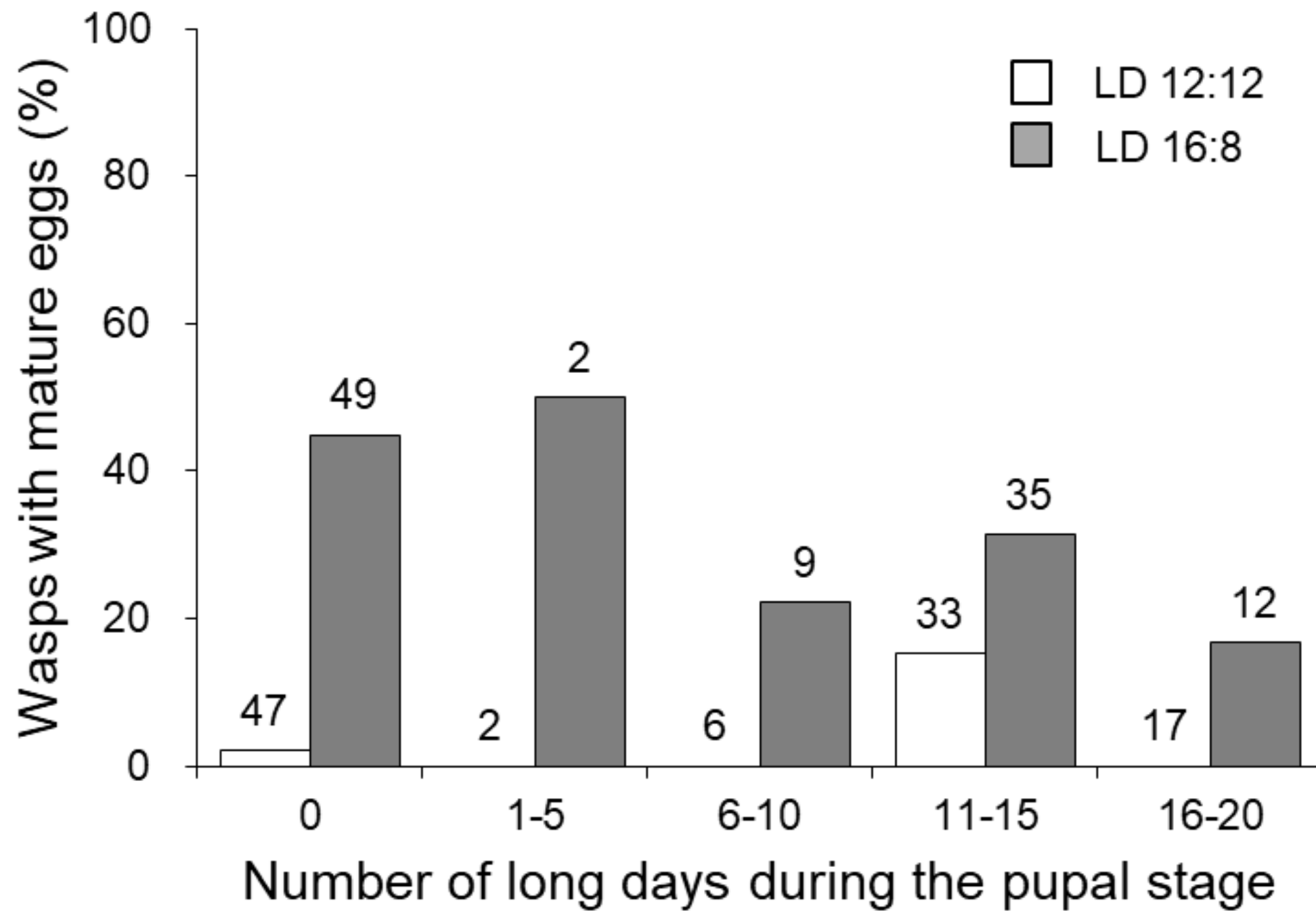


Fig. 5

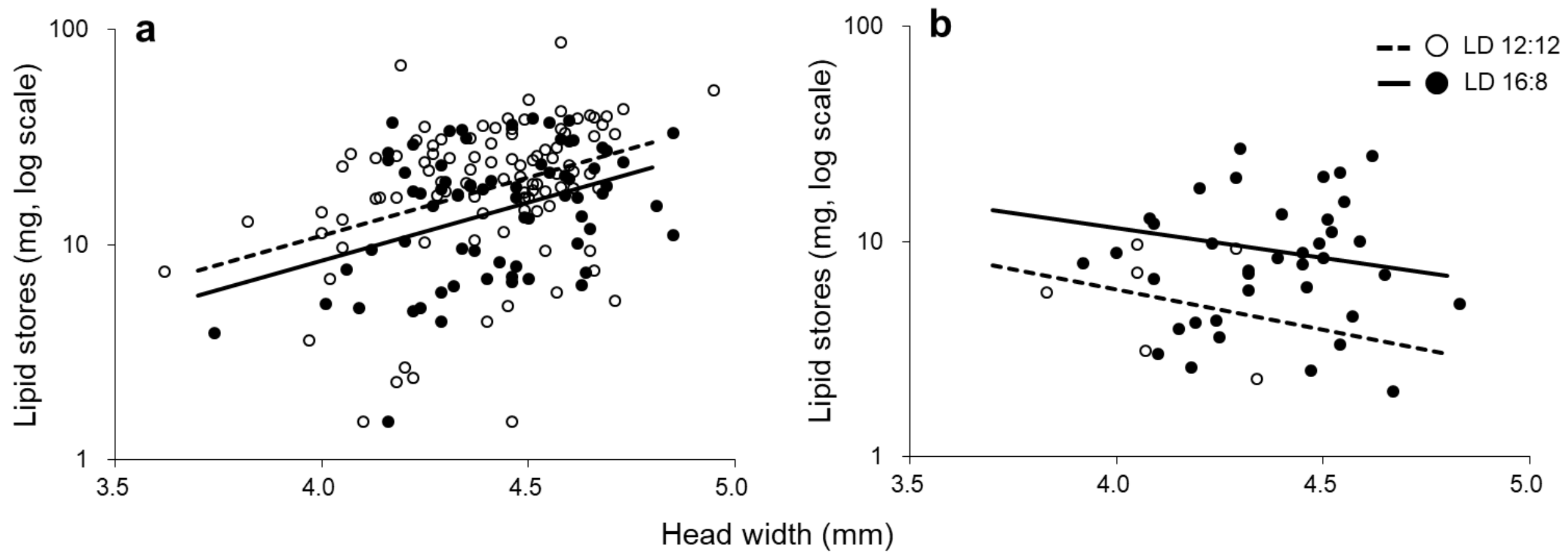


Table 1 Results of statistical analyses of the proportion of female adults with mature eggs: *P*-values for each factor and interaction

| Factor ^a | Variables used for the photoperiod during the immature stage ^b | | | | 0–8 | >8 | Short | Long |
|---------------------|---------------------------------------------------------------------------|--------------------|--------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | L-SH | P-SH | L-L | P-L | long | long | days | days |
| | | | | | days | days | during | during |
| | | | | | during | during | adult | adult |
| | | | | | pupal | pupal | stage ^d | stage ^d |
| | | | | | stage ^c | stage ^c | | |
| HW | 0.001 | <0.001 | 0.001 | <0.001 | 0.293 | <0.001 | 0.005 | 0.022 |
| A | <0.001 | <0.001 | <0.001 | 0.011 | <0.001 | 0.032 | — | — |
| Im | 0.720 | 0.266 | 0.692 | 0.002 | — | — | 0.096 | 1.000 |
| HW*A | 0.116 | 0.112 | 0.114 | 0.004 | 0.112 | 0.034 | — | — |
| HW*Im | 0.730 ^e | 0.383 ^e | 0.308 | 0.060 ^e | — | — | 1.000 | 0.092 |
| A*Im | 0.059 | 0.063 | 0.286 | <0.001 | — | — | — | — |

^a HW, head width; A, photoperiod during the adult stage; Im, photoperiod during the immature stage

^b L-SH, number of short days during the larval stage; P-SH, number of short days during the pupal stage; L-L, number of long days during the larval stage; P-L, number of long days during the pupal stage

^c Analysis was performed separately for wasps with 0–8 and >8 long days during the pupal stage. The P-L was used for the Im

^d Analysis was performed separately for short and long days during the adult stage. The P-L was used for the Im

^e These *P*-values were calculated using parametric methods; the other *P*-values were calculated using exact methods

Table 2 Results of statistical analyses of lipid stores in the gaster

| Factor ^a | All wasps ^b | | | Wasps with mature eggs ^c | | | Wasps without mature eggs ^c | | |
|---------------------|------------------------|----------|----------|-------------------------------------|---------|----------|----------------------------------------|----------|----------|
| | <i>F</i> | DF | <i>P</i> | <i>F</i> | DF | <i>P</i> | <i>F</i> | DF | <i>P</i> |
| HW | 5.2 | 1, 202.4 | 0.023 | 1.7 | 1, 40.4 | 0.194 | 24.4 | 1, 161.4 | <0.001 |
| A | 5.0 | 1, 198.3 | 0.026 | 2.2 | 1, 37.9 | 0.194 | 6.2 | 1, 160.2 | 0.014 |
| EM | 5.1 | 1, 201.3 | 0.025 | — | — | — | — | — | — |
| P-L | 1.7 | 1, 115.3 | 0.197 | — | — | — | — | — | — |
| HW*A | 0.3 | 1, 191.3 | 0.615 | 0.2 | 1, 39.5 | 0.644 | 0.01 | 1, 151.6 | 0.893 |
| HW*EM | 6.3 | 1, 201.1 | 0.013 | — | — | — | — | — | — |
| HW*P-L | 0.3 | 1, 200.9 | 0.561 | — | — | — | — | — | — |
| A*EM | 2.5 | 1, 197.1 | 0.113 | — | — | — | — | — | — |
| A*P-L | 0.3 | 1, 191.9 | 0.573 | — | — | — | — | — | — |
| EM*P-L | 2.3 | 1, 197.2 | 0.128 | — | — | — | — | — | — |

^a EM, with or without mature eggs^b Analysis was performed for all female wasps^c Analysis was performed separately for female wasps with and without mature eggs