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SHORT COMMUNICATION

Keeping a low profile: small hive beetle reproduction in African honeybee colonies

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Abstract

1 Small hive beetles (SHBs) *Aethina tumida* are parasites of honeybee colonies native to sub-Saharan Africa and have become an invasive species.
2 SHB mass reproduction can destroy entire host colonies, although it is very rare in populations of African honeybee subspecies. However, there are no data available on SHB cryptic low-level reproduction in African host colonies.
3 In the present study, we dissected entire African honeybee (*Apis mellifera adansonii*) colonies in Benin.
4 The data obtained show that nondestructive, low-level SHB reproduction can be very common in Africa and is sufficient to explain local infestation levels of host colonies with adult SHBs.

Keywords *Aethina tumida*, *Apis mellifera adansonii*, honeybee, reproduction, small hive beetle.

Introduction

Small hive beetles (SHBs) *Aethina tumida* Murray (Coleoptera: Nitidulidae) are parasites of social bee colonies native to sub-Saharan Africa and have become a widespread invasive species at a global scale (Neumann *et al.*, 2016). SHB reproduction in association with honeybee colonies *Apis mellifera*, their apparent primary hosts, can comprise mass reproduction, often with thousands of larvae (Neumann & Elzen, 2004). This can regularly result in the full structural collapse of the entire nest (Hepburn & Radloff, 1998) and is the main cause for damage to colonies of European honeybee subspecies in the new ranges of the SHB (Neumann *et al.*, 2016). However, SHB mass reproduction is extremely rare in colonies of African honeybee subspecies (Neumann, 2017). Moreover, SHB reproduction in abandoned nests of African honeybee subspecies is two orders of magnitude lower compared with nests of European ones (Neumann *et al.*, 2018). Alternatively, SHB cryptic low-level reproduction in association with honeybees can also occur with only few larvae in the debris of colonies (Spiewok & Neumann, 2006) or underneath sealed honey frames (Neumann & Hoffmann, 2008). This mode of reproduction is nondestructive to the host colonies.

The question emerges as to whether the comparatively low SHB infestation levels of African honeybee colonies (Spiewok *et al.*, 2007) could be explained by cryptic low-level reproduction. Given that this is the case, one would expect a sufficient number of SHB larvae in host colonies to explain the local adult beetle population. However, at present, there are no data available on SHB low-level reproduction in African honeybee colonies. In the present study, we investigated SHB reproduction in African honeybee populations by dissecting entire colonies.

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Materials and methods

In the local dry season (December 2016), when local honeybee colonies are usually declining in bee numbers, samples were collected in two savannah apiaries located approximately 2 km apart in Benin, with naturally SHB-infested local *Apis mellifera adansonii* colonies kept in traditional bark hives (Anguaradebou-Centre A: 11.323293 N, 3.041346 E: $n = 20$; Anguaradebou-Itya B: 11.356610 N, 3.058919 E: $n = 22$). In Benin, beekeepers simply catch swarms. After sunset, 10 colonies per apiary were randomly selected, sealed and killed by pouring 500 mL of petrol into each hive. Then all hives were sealed and transported to the laboratory, where they were stored in a cool room at 10 °C and thoroughly dissected within 3 days to assess the number of SHB larvae and adults. This dissection included the uncapping of all honey and brood frames using an uncapping tool as in routine beekeeping management (Neumann & Hoffmann, 2008) (Table 1).

Table 1 Collected small hive beetle (SHB) life stages after dissection of 20 colonies at savannah apiaries in Benin, showing apiary name and hive number, as well as numbers of SHB larvae and adults

		Life stages of collected small hive beetles	
Apiary	Hive	Total larvae	Total adults
Anguaradebou-Centre	1	69	32
	2	0	0
	3	27	29
	4	0	0
	5	0	0
	6	0	0
	7	23	15
	8	35	17
	9	0	0
	10	0	0
Anguaradebou-Itya	11	24	16
	12	0	0
	13	0	0
	14	32	22
	15	0	0
	16	20	11
	17	0	0
	18	0	0
	19	17	15
	20	21	13
Total	20	268	170

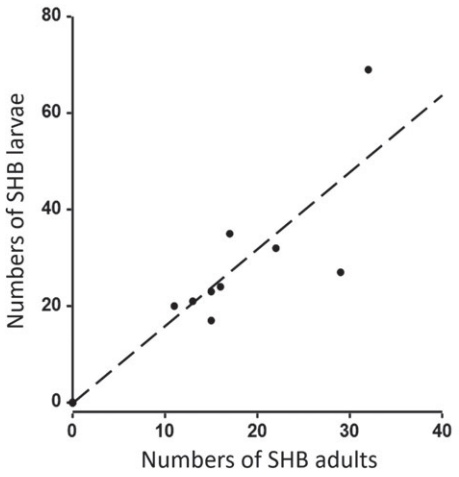

Figure 1 Correlation between small hive beetle (SHB) *Aethina tumida* Murray (Coleoptera: Nitidulidae) larvae and adults present within 20 dissected honeybee colonies in Benin. A significant positive correlation was observed between SHB larvae and adults present within an infested colony (Pearson correlation, $|r| = 0.9334$, d.f. = 18, $P < 0.001$).

All tested variables were normally distributed (Shapiro–Wilk's test, $P > 0.05$). A chi-squared test was used to compare infestation rates between the apiaries. A one-way analysis of variance (ANOVA) was used to compare numbers of SHB larvae and adults between colonies and apiaries. The existence of an association between measured variables (e.g. SHB larvae and SHB adults) was assessed by Pearson's correlation coefficients (Fig. 1).

Results and Discussion

All colonies were queenright and had brood of various stages. The overall SHB infestation rate of the colonies was 45% and each SHB infested colony had both larvae and adults present. In total, 268 SHB larvae (29.78 ± 15.78 per colony; mean ± SD) and 170 SHB adults were found (13.67 ± 4.06 per colony). As a result of the destructive sampling method, which was inevitable in the traditional bark hives, it was not possible to determine whether the SHB larvae and/or adults were located on the combs or in the debris. However, none of the colonies showed any clinical symptoms of SHB mass reproduction, including slimy and/or partially destroyed combs (Neumann & Elzen, 2004). Therefore, it appears more likely that the larvae were in the debris and not on the combs. Indeed, no SHB larvae were found underneath any of the dissected honey and brood combs. There were no significant differences between apiaries with respect to infestation rates ($\chi^2 = 0.127$, d.f. = 1, $P = 0.72$), number of SHB larvae (15.4 ± 23.27 in apiary 1; 11.4 ± 12.61 in apiary 2; mean ± SD) (one-way ANOVA, $F_{1,20} = 0.228$, $P = 0.64$) and SHB adults (9.3 ± 12.97 in apiary 1; 7.7 ± 8.56 in apiary 2; mean ± SD) (one-way ANOVA, $F_{1,20} = 0.11$, $P = 0.75$), although there were significantly more SHB larvae present than adults (one-way ANOVA, d.f. = 1, $F_{1,20} = 3.52$, $P = 0.001$). There was a significant positive correlation between SHB larvae and adults within colonies (Pearson's correlation, $|r| = 0.93$, d.f. = 18, $P < 0.001$).

The data suggest that nondestructive, cryptic low-level reproduction alone can be sufficient to explain infestation levels of African honeybee colonies with adult SHBs.

The observed low adult SHB infestation levels in the savannah of Benin compared with Nigeria (51 ± 44.07 adults per colony, Kayode & Neumann, 2018) could be a result of seasonal differences (wet versus dry season, Lawal & Banjo, 2008). Moreover, the experimental colonies were exposed to sunlight, which may have also contributed because adult SHBs prefer colonies in shaded locations (Arbogast *et al.*, 2009).

Because 100% of SHB-infested colonies also had SHB larvae, this suggests that low-level reproduction can be very common in *A. m. adansonii* colonies under the given conditions. By contrast, only 6.25% of European-derived honeybee colonies in the U.S.A. (Spiewok & Neumann, 2006) and 10% in Australia (Neumann & Hoffmann, 2008) displayed cryptic low-level SHB reproduction. The ample debris in the bark hives may have fostered both the chance and magnitude of SHB low-level reproduction in the test colonies. The positive correlation between SHB larvae and adults within infested colonies further suggests that the magnitude of SHB low-level reproduction may be influenced by adult infestation levels of colonies. Even if a single SHB female could have potentially produced all larvae found in the entire study by her own (Neumann *et al.*, 2016), the actual oviposition choice in the debris may have been triggered by some occasional food (e.g. dead bees), which is more likely to be detected under higher adult infestation levels. Interestingly, the numbers of SHB larvae in colonies were significantly larger than the numbers of adults. Therefore, losses as a result of the hosts (Neumann & Härtel, 2004), natural enemies (Torto *et al.*, 2010) or unfavourable conditions for pupation (Neumann *et al.*, 2016) could possibly be compensated for. In conclusion, it appears as if the observed low-level reproduction can be sufficient to explain local infestation levels of African honeybee colonies with adult

SHBs irrespective of any other reproductive options (Neumann *et al.*, 2016).

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