

ORIGINAL ARTICLE

Gregarious pupation act as a defensive mechanism against cannibalism and intraguild predation

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Abstract Coccinellid pupae use an array of defensive strategies against their natural enemies. This study aims to assess the efficiency of gregarious pupation as a defensive mechanism against intraguild predators and cannibals in coccinellid. The study was designed specifically (i) to determine the natural occurrence of gregarious pupation in the field for different coccinellid species, and (ii) to evaluate the adaptive value of gregarious pupation as a defensive mechanism against 2 types of predators (i.e., cannibals and intraguild predators). In the field, gregarious pupation consisted of a group of 2–5 pupae. The proportion of gregarious pupation observed varied according to species, the highest rate being observed with *Harmonia axyridis* Pallas (Coccinellidae; 14.17%). Gregarious pupation had no impact on the probability that intraguild predators and cannibals locate pupae. Intraguild predation occurred more often in site with gregarious pupation, while cannibalism occurred as often in site with gregarious pupation as in site with isolated pupa. However, for a specific pupa, the mortality rate was higher for isolated pupae than for pupae located in a gregarious pupation site both in the presence of intraguild predators and in the presence of cannibals. The spatial location of pupae within the group had no impact on mortality rate. Since it reduces the risk of predation, it is proposed that gregarious pupation act as a defensive mechanism for *H. axyridis* pupae.

Key words attack abatement; Coccinellidae; dilution effect; encounter effect; grouping behavior; Pentatomidae; Selfish herd

Introduction

Like in most holometabolous insects, ladybeetle (Coleoptera: Coccinellidae) pupae are sessile and thus vulnerable to natural enemies. Pupae may be victims of parasitoids that attack all coccinellid life stages, and some dipteran parasitoids, such as *Phalacrotophora* sp. (Diptera: Phoridae), are even late prepupal or pupal parasitoids specialists (Osawa, 1992; Hurt *et al.*, 1998; Kenis *et al.*, 2008; Riddick *et al.*, 2009). Ladybeetle pupae may also be preyed upon by conspecific ladybeetle larvae or by

intraguild predators (Osawa, 1992; Hironori & Katsuhiko, 1997; Sakuratani *et al.*, 2000; Félix & Soares, 2004; Lucas, 2005, 2012). Many coccinellids species have, however, developed different morphological (gin traps, Eisner & Eisner, 1992; glandular setae, Attygalle *et al.*, 1993; and hairs, Schroeder *et al.*, 1998; Smedley *et al.*, 2002; coarse setae, Montgomery *et al.*, 2002; Lu *et al.*, 2002; and behavioral, flipping, Lucas, 2005, 2012; moving away from host aphid colony, Osawa, 1992; Lucas *et al.*, 2000) mechanisms to overcome this pupal vulnerability. Another intriguing characteristic about coccinellid pupae is that they are sometimes observed to be clumped. However, the reason for this behavior is unknown. One possible explanation could be that gregarious pupation is a defensive strategy that helps reducing predation through different possible mechanisms.

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The protective effect provided by grouping arise from a simple dilution effect, where the bigger a group is, the lower is the probability for a given individual to fall to predation (Reaka, 1976; Foster & Treherne, 1981; Wcislo, 1984; Turchin & Kareiva, 1989; Lucas & Brodeur, 2001; DeVito, 2003; Aukema & Raffa, 2004). For example, Turchin and Kareiva (1989) demonstrated that the probability for an individual aphid *Aphis varians* Patch (Hemiptera: Aphididae) to be preyed upon by *Hippodamia convergens* Guérin-Méneville is inversely proportionate to the size of its colony through a dilution effect. Wcislo (1984) also showed that the proportion of *Crabro cribrellifer* (Packard) (Hymenoptera: Sphecidae) cells parasited by *Metopia campestris* (Fallén) (Diptera: Sarcophagidae) was lower as nest density increases. However, predators may more readily detect larger group than smaller ones. For example, Ioannou *et al.* (2009) demonstrated that the predator *Gasterosteus aculeatus* L. (Gasterosteiformes: Gasterosteidae) triggered attack more frequently on denser part of *Daphnia magna* Straus (Cladocera: Daphniidae) groups than expected, suggesting that density of prey attracts the attention of predators. That is, detection may not be enhanced proportionately to group size enhancement. The term encounter effect is used when the probability that a colony is detected does not increase proportionately to group size. Thus, grouping is advantageous for an individual when the benefits of herding (dilution effect) outweigh the costs (encounter effect), a trade-off referred as attack abatement (Turner & Pitcher, 1986; Turchin & Kareiva, 1989; Jensen & Larsson, 2002). For example, Watt *et al.* (1997) have shown that bigger groups of *Bufo bufo* (L.) (Anura: Bufonidae) tadpoles suffer higher attack rate by the predator *Carassius auratus* (L.) (Cypriniformes: Cyprinidae). However, the attack rate on individual tadpole lowers as the groups gets larger. Also, it has been shown that *Rhyacophila vao* Milne pupae (Trichoptera: Rhyacophilidae) are more encountered by the predator *Polycelis coronata* (Girard) (Tricladida: Planariidae) when in a larger group, but individuals are less susceptible in larger groups due to a dilution effect; when the 2 factors are combined, an inverse relation is still observed between group size and mortality risk, suggesting that herding is advantageous against predation (Wrona & Dixon, 1991).

It has also been proposed that position of an individual within a group has an influence on its susceptibility to predation. The selfish herd hypothesis assumes that a predator attacks the nearest prey, and that this establishes a domain of danger for each individual prey (Hamilton, 1971). For an animal, this domain of danger includes any area closer to him than to any of its conspecific. Grouping animal behavior may thus evolve if the animals

selfishly lower their predation risks by reducing their domain of danger by moving toward conspecifics. One implication of this theory is that the domain of danger, and thus predation risk, should be lower for individuals located in the center of the herd than for those located at the periphery. This theory has been indirectly tested on minnows by Krause (1993), and Rayor and Uetz (1990) demonstrated that within colonies of the colonial spider *Metopeira incrassata* F.O. Pickard-Cambridge (Araneae: Araneidae), individuals located at the periphery are more susceptible to predation than those located in the center. Some experiments suggest that even peripheral individuals of two dimensionally distributed colonies should be more susceptible to predation by three-dimensional roaming predators (Romey *et al.*, 2008). Currently there is no information available about the possible existence of selfish herd type mechanisms in larvae, pupae, or adult ladybeetles. Adults of many species are known to be gregarious during hibernation but the reasons for this behavior is still unknown (Honěk *et al.*, 2007). Also, gregarious pupation is sometime observed.

The objectives of this study were thus (i) to evaluate the field occurrence of gregarious pupation in Coccinellidae, (ii) to evaluate the defensive value of gregarious pupation against natural enemies, and (iii) to evaluate if the location of a pupa within an aggregation can modulate the efficacy of the defensive effect.

Materials and methods

Biological material

Harmonia axyridis individuals used in this experiment came from a colony established from field caught ladybeetles. Larvae and adults were fed with a mix of potato aphids *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), pollen, and *Sitotroga cerealella* (Lepidoptera: Gelechiidae) eggs. Adults also had access to sugar water. Some pupae used for the experiments came directly from 3rd or 4th instar larvae caught in the field. *Podisus maculiventris* Say (Hymenoptera: Pentatomidae) was purchased as eggs from Plant-Prod Quebec (Laval, Quebec, Canada). Nymphs were raised on a diet of mealworm, potato aphids, and sugar water. Adults were used for the experiments.

Experiment 1—Field observations of gregarious pupation

Observations were done from 2007 to 2009 in 6 organic soybean fields on a farm located at Les Cèdres,

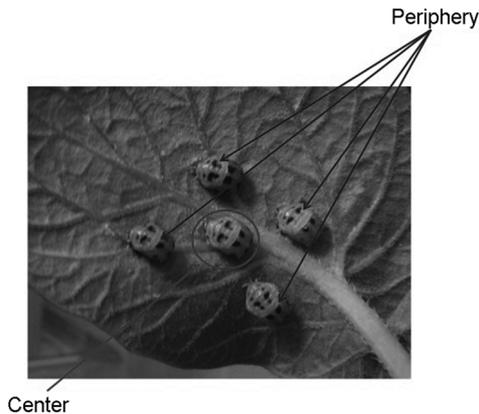


Fig. 1 Picture of *Harmonia axyridis* gregarious pupation site on a potato leaf during intraguild predation and cannibalism tests (Exp. 2).

near Montreal (Quebec, Canada). Three 60-m transects were established in each field and observations were done each week between June and September. All pupae that could be spotted while walking along transects were noted. Moreover, 6 plants by transect were thoroughly inspected. All pupae were identified to the species level by keeping pupae to laboratory until emergence of adult. It was also noted whether the pupae were alone or in group (2 or more pupae on the same leaf). When in group, the number of pupae was noted.

Experiment 2—Impact of gregarious pupation on predation susceptibility

Laboratory experiments were done in order to evaluate the defensive value of gregarious pupation against intraguild predators and cannibals. Pupae of *H. axyridis* were used because this species shows the highest occurrence of gregarious pupation in the field (see results of experiment 1).

Harmonia axyridis pupae (aged 24–72 h) attached on potato saplings were exposed to natural enemies for 24 h. Pupae were either presented (i) alone (solitary) or (ii) in a group of 5 (gregarious) on potato saplings (largest group observed in the field counted 5 pupae; see result of experiment 1). In order to obtain those pupae, 2nd or 3rd instar larvae were raised individually in Petri dishes. Once pupal stage was reached, each pupa was gently removed with soft pincers and glued on the underside of a potato sapling leaf with nontoxic glue. Groups were arranged in a way that 4 pupae formed a square (peripheral pupae) and 1 pupa in the center of the square (central pupae) (Fig. 1). This manipulation had no negative impact on the gin trap

mechanism. In control tests (without predators), all pupae develop to adult without presenting malformations.

The potato saplings used (3–4 week old) were standardized to 5 leaves. All leaflets and the apex were removed, resulting in sapling of about 15–25 cm in height.

The predators used were *H. axyridis* 4th instar larvae (cannibalism) and *P. maculiventris* adults (intraguild predation). Predators were starved for 24 h before the beginning of the experiment. Predators were introduced with a fine hairbrush on the stem (2.5–3.0 cm from the ground) of the experimental plant, with the head directed toward the upper part of the plant.

Continuous observations were made during the first hour, where it was noted whether or not the predator contacted a pupa, the time elapsed before the first contact with a pupa and the number of flipping made by pupae when contacted. Predators and pupae remained together for a further 23 h. Mortality was measured 24–72 h after the predator and the pupae have been separated. Mortality measures included both killed (no adult emergence) and adults emerging with malformations. Moreover, in order to determine whether the position within the group has an influence on predation susceptibility, the position of the pupa that was preyed upon in the gregarious treatment was noted. From these observations, we calculated 3 parameters related to predator's activity: (i) Localization of the pupation site by the predator (contact predator-pupa); (ii) Predation at pupation site (i.e., at least 1 pupa killed by the predator); (iii) Individual pupal mortality rate (mean mortality of a pupa).

It was predicted that predators should not find groups of pupae more rapidly than isolated pupae, but that individual mortality should be greater for isolated pupae than for pupae located in a group.

Statistical analysis

Experiment 1: We first determined the probability of gregarious pupation to occur randomly using a Monte Carlo simulation. The probability to observe gregarious pupae on a leaf depends on the number of pupae and the number of leaves available to pupation (e.g., if there is 2 pupae and 100 leaves, the probability to observe gregarious pupation on a leaf is 0.0001). We observed a total of 605 pupae in the 6 fields we sampled. Since we do not know the exact number of leaves in those fields, we ran the Monte Carlo simulation for a range of number of leaves varying from 1000 to 100 000. We first randomly attribute a pupation site (a leaf) to each pupa ($n = 605$). We then determine the probability to observe gregarious pupation (≥ 2 pupae) on a single pupation site by dividing

the number of leaf with 2 pupae and more by the total number of leaf. We ran 1000 iterations of this procedure and retained the highest probability for each number of leaf. If the probabilities to observe gregarious pupation on a pupation site fall under 0.05, we rejected the null hypothesis that gregarious pupation occurs only randomly. Moreover, the probabilities associated with each species level of gregarious pupation were calculated by dividing the number of time the simulation reach the observed % of gregarious pupation of each species by the number of iteration (1000).

We then compared gregarious pupation among the 3 species in which gregarious pupation was observed (i.e., *Harmonia axyridis*, *Coleomegilla maculate*, and *Coccinella septempunctata*). We implemented zero-truncated Poisson model (Zuur et al., 2009) vglm function of VGAM package (Yee, 2013) in R software (R Core Team, 2012). The model included pupae species and species density (number of pupae of a specific species observed in a field) as explanatory variables of the number of pupae in a single pupation site ($n = 519$).

Experiment 2: For each predator species, (i) the proportions of pupation sites contacted by predators, (ii) the proportions of pupation site where predation occurred, and (iii) the individual pupal mortality rate were compared with likelihood ratio tests.

Results

Experiment 1 – Field observations of gregarious pupation

A total of 605 pupae (434 *H. axyridis*, 70 *C. maculata*, 82 *C. septempunctata*, 11 *H. convergens*, 8 *P. quatuordecimpunctata*) were observed on 538 pupation sites (leaves). For such a pupal density, the probability to randomly observe a gregarious pupation (≥ 2 pupae) (0.037) is under the alpha 0.05 when 2000 leaves or more are available in the field (Fig. 2). In such situation, the null hypothesis that gregarious pupation occurs only by chance in coccinellid is rejected.

Gregarious pupation was observed in 14.17% of *Harmonia axyridis* pupae, and in 4.55% and 3.80% of *Coleomegilla maculata* and *Coccinella septempunctata* pupae, respectively (Fig. 3). Considering the density of these 3 species, the probabilities to randomly observe their respective level of gregarious pupation were null ($P = 0$) in a field containing 1000 leaves or more. Gregarious pupation did not occur randomly in *H. axyridis*, *C. maculata*, and *C. septempunctata*. However, gregarious pupation was not observed in *Hippodamia convergens*

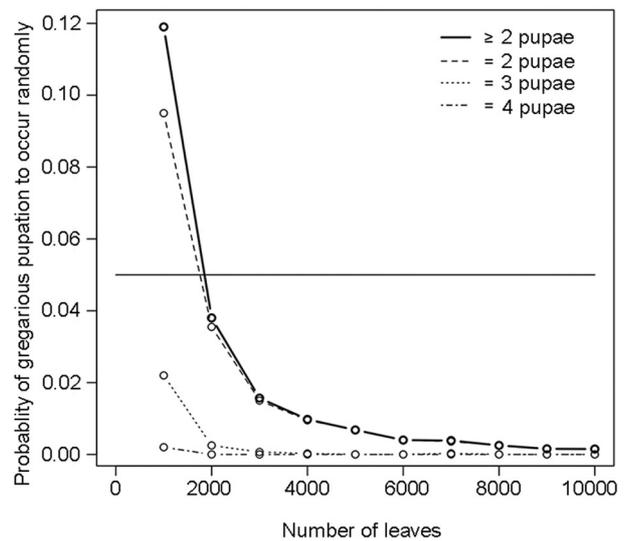


Fig. 2 Probability of gregarious pupation (2 pupae or more on the same leaf) to randomly occur in function of the number of leaves in the sampled area. The horizontal full line indicates a probability of 0.05.

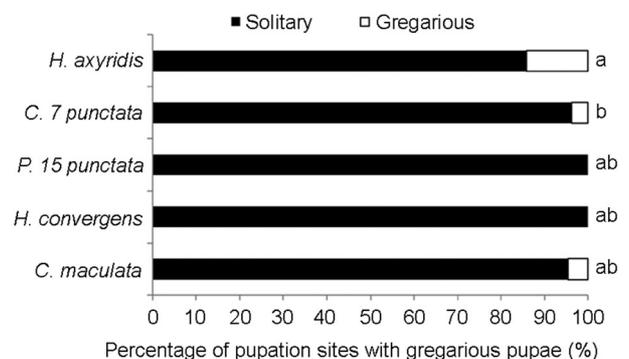


Fig. 3 Percentage of pupation sites with gregarious pupae (n : *H. axyridis* = 374; *C. septempunctata* = 79; *P. quatuordecimpunctata* = 9; *H. convergens* = 10; *C. maculata* = 66). Species with the same letter are not significantly different from each other.

and *Propylea quatuordecimpunctata*. On one occasion, gregarious pupation consisted of 1 *H. axyridis* pupa with 1 *C. maculata* pupa.

Of the 53 *H. axyridis* pupation sites observed in a gregarious situation, 48 (90.57%) contained 2 pupae, 4 (7.54%) contained 3 pupae, and 1 (1.89%) contained 5 pupae. In *C. maculata*, 2 gregarious situations out of 3 (66.7%) contained 2 pupae. In one occasion, we observed 3 *C. maculata* pupae aggregated. All the gregarious situations (3) in *C. septempunctata* contained 2 pupae.

Gregarious pupation was more frequent in *H. axyridis* (1.56 ± 0.60 ; $z = 2.59$; $P = 0.005$) than in *C. maculata*

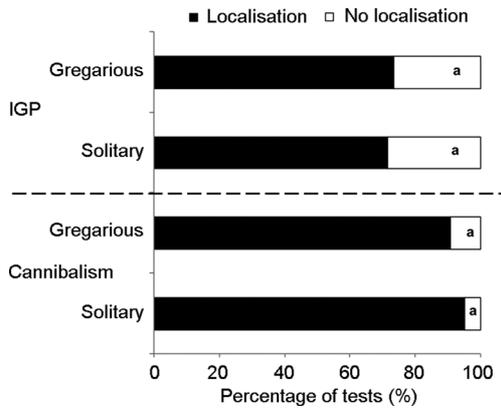


Fig. 4 Percentage of tests where a pupa has been localized in less than 1 h by the intraguild predator *Podisus maculiventris* (IGP) (n : solitary pupa = 10; gregarious pupae = 24) or by the cannibal *Harmonia axyridis* (Cannibalism) (n : solitary pupa = 20; gregarious pupae = 22). For the same type of interaction (IGP or Cannibalism), result with the same letter indicate that gregarious and solitary situations are not different.

and *C. septempunctata* (0.45 ± 0.76 ; $z = 0.59$; $P = 0.28$). The population density of the pupae species did not had a significant effect on gregarious pupation (-0.002 ± 0.002 ; $z = -1.17$; $P = 0.12$).

Experiment 2 – Impact of gregarious pupation on predation susceptibility

Intraguild predation: *Podisus maculiventris* adults localized *H. axyridis* pupae in less than an hour in 73.33% of the tests of the gregarious treatment ($n = 24$) and in 71.43% of the isolated treatment ($n = 10$) (Fig. 4): the difference between those treatments was not significant (likelihood ratio: $khi^2 = 0.03$; $df = 1$; $P = 0.87$). Even though the mean number of flipping observed was slightly higher for individuals located in the gregarious treatment ($16.53\% \pm 9.37\%$) than in the isolated treatment ($12.79\% \pm 6.50\%$), the difference was not significant (Fig. 5; Wilcoxon: $khi^2 = 0.66$; $df = 1$; $P = 0.42$).

Intraguild predation occurred in 100% of the tests in the gregarious treatment and in 80.00% of the tests in the isolated treatment (Fig. 6). This difference was significant (likelihood ratio: $khi^2 = 7.28$; $df = 1$; $P = 0.007$). For a specific pupa, the mortality rate was significantly higher in the isolated treatment ($80.00\% \pm 8.17\%$) than in the gregarious treatment ($45.00\% \pm 5.28\%$) (Fig. 7; Wilcoxon: $khi^2 = 12.70$; $df = 1$; $P = 0.0004$).

The first pupa contacted was the central pupa in 13.64% of the observation and a peripheral pupa in 86.36% of the observation (Fig. 8). This was not significantly different

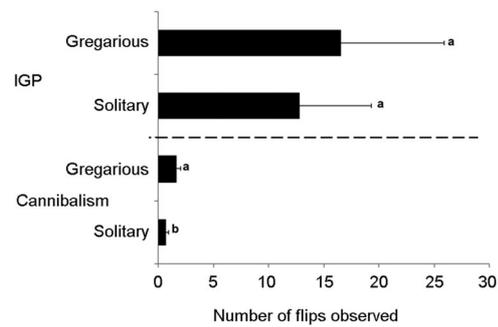


Fig. 5 Number of flips observed during a 1-h period in gregarious and solitary pupation sites in the presence of the intraguild predator *Podisus maculiventris* (IGP) (n : solitary pupa = 10; gregarious pupae = 24) or of the cannibal *Harmonia axyridis* (Cannibalism) (n : solitary pupa = 20; gregarious pupae = 22). For the same type of interaction (IGP or Cannibalism), result with the same letter indicate that gregarious and solitary situations are not different.

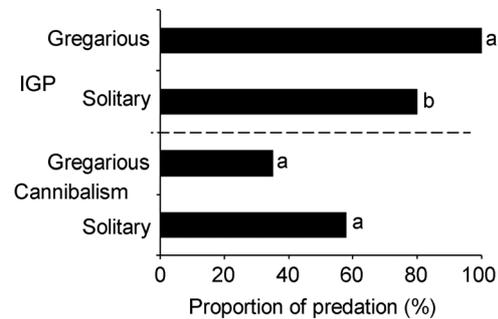


Fig. 6 Proportion of tests with occurrence of predation by the intraguild predator *Podisus maculiventris* (n : solitary pupa = 10; gregarious pupae = 24) or by the cannibal *Harmonia axyridis* (n : solitary pupa = 20; gregarious pupae = 22). For the same type of interaction (IGP or Cannibalism), result with the same letter indicate that gregarious and solitary situations are not different.

from the theoretical 20%–80% probability of encounter (likelihood ratio: $khi^2 = 0.61$; $df = 1$; $P = 0.43$). The mortality rate of central pupae was $45.83\% \pm 10.39\%$, while it was $43.75\% \pm 5.27\%$ for peripheral pupae (Fig. 9). This difference was not significant (Wilcoxon: $khi^2 = 0.12$; $df = 1$; $P = 0.73$).

Cannibalism

Harmonia axyridis larvae localized *H. axyridis* pupae in less than an hour in 90.91% of the tests of the gregarious treatment ($n = 22$) and in 95.00% of the isolated treatment ($n = 20$) (Fig. 4): the difference between these treatments were not significant (likelihood ratio: $khi^2 = 0.27$;

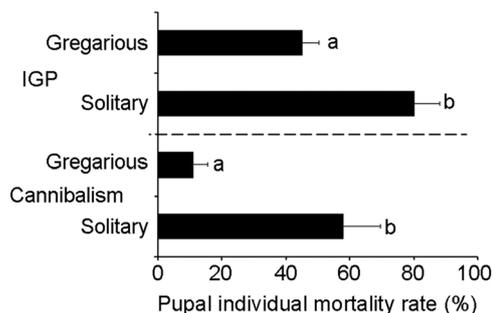


Fig. 7 Pupal individual mortality rate within solitary or gregarious pupation sites in the presence of the intraguild predator *Podius maculiventris* (IGP) (n : solitary pupa = 10; gregarious pupae = 24) or of the cannibal *Harmonia axyridis* (Cannibalism) (n : solitary pupa = 20; gregarious pupae = 22). For the same type of interaction (IGP or Cannibalism), result with the same letter indicate that gregarious and solitary situations are not different.

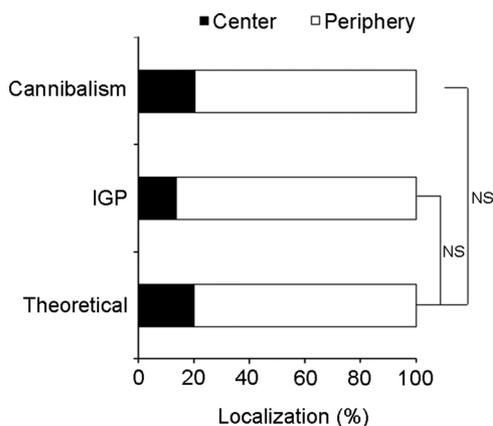


Fig. 8 Localization (center or periphery) of the first pupa encountered by the cannibal *Harmonia axyridis* (Cannibalism) ($n = 22$) or by the intraguild predator *Podisus maculiventris* (IGP) ($n = 24$) and comparison to the theoretical proportions (20% in the center).

$df = 1$; $P = 0.60$). The mean number of flipping observed was higher for individuals located in the gregarious treatment ($1.62\% \pm 0.44\%$) than in the isolated treatment ($0.65\% \pm 0.30\%$) (Fig. 5). The difference was significant (Wilcoxon: $khi^2 = 5.12$; $df = 1$; $P = 0.03$).

Cannibalism occurred in 35.00% of the tests in the gregarious treatment and in 57.89% of the tests in the isolated treatment (Fig. 6). Unlike intraguild predation, this difference was not significant (likelihood ratio: $khi^2 = 2.073$; $df = 1$; $P = 0.15$). For a specific pupa, the mortality rate was higher in the isolated treatment ($57.89\% \pm 11.64\%$) than in the gregarious treatment ($11.00\% \pm 4.47\%$) (Fig. 7; Wilcoxon: $khi^2 = 6.48$; $df = 1$; $P = 0.01$).

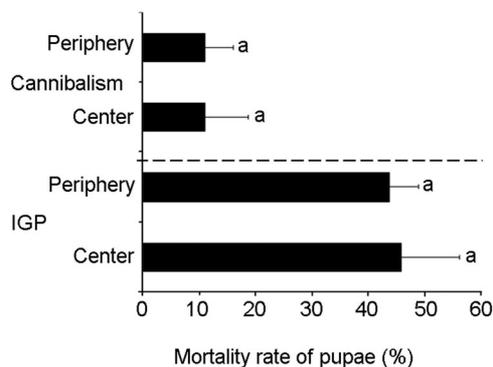


Fig. 9 Mortality rate of pupae localized in central or peripheral zone of the aggregation in the presence of the cannibal *Harmonia axyridis* (Cannibalism) ($n = 22$) or of the intraguild predator *Podisus maculiventris* (IGP) ($n = 24$). For the same type of interaction (IGP or Cannibalism), result with the same letter indicate that gregarious and solitary situations are not different.

The first pupa contacted was the center pupa in 15% of the observation and a peripheral pupa in 85% of the observation (Fig. 8). This was not significantly different from the theoretical 20%–80% probability of encounter (likelihood ratio: $khi^2 = 0.34$; $df = 1$; $P = 0.56$). The mortality rate of central pupae was $11.11\% \pm 7.62\%$, while it was $11.11\% \pm 5.04\%$ for peripheral pupae (Fig. 9). This difference was not significant (Wilcoxon: $khi^2 = 1.02$; $df = 1$; $P = 0.31$).

Discussion

The first aim of this experiment was to determine the occurrence of gregarious pupation (here defined as the presence of 2 or more pupae on the same leaf) in the field. Observations revealed that gregarious pupation is relatively common in some coccinellid species, especially *H. axyridis*. Aggregations of 2 or more pupae were observed more often than what would be expected if individuals chose pupation sites randomly. This means that, in the field, coccinellid individuals make the decision to pupate close to already present pupae. The reason for this behavior yet remains to be explained. It could be a consequence of a high coccinellid density and/or a consequence of a great synchronicity of 4th instar larvae (perhaps in order to reduce the risk of cannibalism), combined with the fact that ladybeetles' larvae tend to aggregate in aphid colonies (Kawai, 1976). The link between synchronicity and predation protection has been documented also for some other animals. For example, Reaka (1976) observed that some stomatopod crustaceans synchronize their molt, possibly as a mechanism of reducing predation risk during

this period of vulnerability. Also, *Bufo americanus* Holbrook (Anura: Bufonidae) tadpoles show more synchrony during metamorphosis when in presence of a chemical cue indicating the presence of a predator (DeVito, 2003). Nevertheless, 4th instar larvae of many coccinellid species, such as *C. maculata* (Lucas *et al.*, 2000) and *H. axyridis* (Osawa, 1992), generally disperse from foraging site before pupation. Thus, a relatively common occurrence of gregarious pupation in nature, such as what was observed with *H. axyridis*, would it be only 2 pupae by leaf, is unlikely to be fortuitous. Our Monte Carlo simulation demonstrated that population density and pupae synchronicity, which are required to achieve gregarious pupation, do not explain alone this phenomenon. Gregarious pupation could thus have evolved for some reason: one hypothesis is that gregarious pupation act as a defensive mechanism against natural enemies.

The second aim of this study was to test the hypothesis that gregarious pupation is an effective defensive mechanism against predation. Our results clearly demonstrate the adaptive value of gregarious pupation behavior as a defense against cannibals and also against intraguild predators. Globally, when considering the aggregation of pupae, events of intraguild predation occurred more often in sites with gregarious pupae, while cannibalism occurred as often in site with gregarious pupae and in site with isolated pupa. However, the individual pupal mortality rate was significantly higher for an isolated pupa than for a pupa located in an aggregation, both in the presence of intraguild predators, and in the presence of cannibals. The spatial location of pupae within the group, however, had no impact on mortality rate, suggesting that pupae located in gregarious pupation sites are protected from intraguild predators and cannibals through a dilution effect, and not through a selfish herd effect. Thus, as in the caddisfly *Rhyacophila vao* (Wrona & Dixon, 1991), dilution effect deriving from pupal aggregation provides net fitness benefits to coccinellid pupae.

In our field study, only 14% of the Asiatic ladybeetles pupated in groups. This should be linked to the probability for a prepupating larva to find another pupae, assuming that gregarious pupation entails prepupating larvae actually search for conspecific pupae. It is thus expected that prepupating larva will balance searching time devoted to finding conspecific pupae (and consequent risk of encountering a predator), with adaptive value provided by protection against cannibals and intraguild predators. Gregarious pupation may have evolved under high population density, when risk of cannibalism increases and searching time for conspecific pupae decreases. For instance, in rearing units, according to the high density and high synchronicity, it is quite common to observe groups

of pupae (Lucas, pers. com). While our results suggested that gregarious pupation is deliberate, it is yet to be tested that coccinellid prepupating larvae actively seek for conspecific pupae. Such behavior may be also influenced by the age of the pupa encountered: a hypothesis would be that pupating near an old pupa (close to emerge as adult) would be more risky than pupating near a recent pupa (risk of cannibalism by emerging adult and shorter duration of gregarious pupation).

Beyond the dilution effect, other factors may favor the emergence of gregarious pupation. Most aphidophagous ladybeetles produce toxic alkaloids. Thus, if a predator can learn through experience to avoid distasteful preys, it is advantageous for those preys to aggregate (Sillén-Tullberg & Leimar, 1988). In such a situation, a group of even only 2 individuals could be advantageous if the predator is reluctant to eat a second distasteful prey after first negative experience. Moreover, ladybeetles, as many aposematic species synthesize pyrazines, which constitute the most widespread alert signals that, can act at distance as a forerunner (Moore *et al.*, 1990; Cai *et al.*, 2007). Therefore, it is possible that aggregation reinforces the signal of pyrazines. Such mechanism has been proposed to explain why eggs of *Coccinella transversalis* (Fabricius) and *Menochilus sexmaculatus* (Fabricius) are better protected against intraguild predation when presented in batch than individually (Agarwala & Yasuda, 2001). However, in the present study, giving that both intraguild predators and cannibals localized evenly isolated and gregarious pupae, it is unlikely that pyrazine signals increased the benefit of gregarious pupation.

It would be interesting to determine if some factors influence the tendency to do gregarious pupation. For example, Orpwood *et al.* (2008) have demonstrated that the *Phoxinus phoxinus* (L.) (Cypriniformes: Cyprinidae) aggregation behavior in response to predator presence is influenced by habitat complexity and occurs only in simple habitat. Other reasons could explain the grouping behavior of ladybeetles. For example, larvae of the sawfly *Perga affinis* Kirby (Hymenoptera: Pergidae) seem to aggregate mainly in order to enhance their development speed through higher temperature (Fletcher, 2009). For *Aphis fabae* Scopoli, Hodgson and Godfray (1999) proposed that aggregation of founder could be the result of patchily distributed highly nutritive area or of diminution of cost associated with new site colonization. Jensen and Larsson (2002) also proposed that patchy distribution of food could explain aggregation of *Daphnia pulex* (L.), even though this aggregation also protects daphnia from predation by *Chaoborus flavicans* (Meigen) (Diptera: Chaoboridae).

Future studies should explore the impact of gregarious pupation on parasitism risk. The impact of gregarious pupation on parasitism has not been studied. However, the dilution effect and the selfish herd theory should apply as well to parasitoids as to predators (Mooring & Hart, 1992; Fauchald, 2007). Also, future studies should explore the factors that influence the tendency to aggregate before pupation in *H. axyridis* and whether or not aggregated pupae are more readily located by predators and parasitoids.

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Disclosure

The authors have no any conflict of interest.

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