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Correspondence and requests for materials should be addressed to A.J. (arne.janssen@uva.nl)

* Current address: Laboratory of Applied Entomology, Department of Horticulture, Chiba University, Matsudo 648, Chiba, 271-8510, Japan.

Predator-prey role reversals, juvenile experience and adult antipredator behaviour

Yasuyuki Choh[1]*, Maira Ignacio[2], Maurice W. Sabelis[1] & Arne Janssen[1]

[1]Institute for Biodiversity and Ecosystem Dynamics, Department of Population Biology, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands, [2]Graduate Programme in Plant Science, Federal University of Tocantins, Gurupi, Brazil.

Although biologists routinely label animals as predators and prey, the ecological role of individuals is often far from clear. There are many examples of role reversals in predators and prey, where adult prey attack vulnerable young predators. This implies that juvenile prey that escape from predation and become adult can kill juvenile predators. We show that such an exposure of juvenile prey to adult predators results in behavioural changes later in life: after becoming adult, these prey killed juvenile predators at a faster rate than prey that had not been exposed. The attacks were specifically aimed at predators of the species to which they had been exposed. This suggests that prey recognize the species of predator to which they were exposed during their juvenile stage. Our results show that juvenile experience affects adult behaviour after a role reversal.

Prey can reduce predation risk in several ways, for example through morphological changes[1–4] or through changes in behaviour[5–10]. They may also protect themselves and their offspring by counterattacking the predators[11–16]. Besides the obvious benefit of reducing predation, antipredator behaviour comes with energetic costs spent in escaping or costs associated with lost feeding and mating opportunities[6,8,17]. It is therefore important to correctly assess predation risk and to mount an appropriate response. Non-lethal encounters with predators or cues associated with predators can result in correct enemy identification, and subsequent encounters with these enemies indeed reinforce antipredator responses in prey[18–22]. However, it is not known whether encounters of young, vulnerable prey with predators still affect the prey's behaviour by the time they have reached an invulnerable older prey stage. For example, juvenile prey could learn to avoid cues associated with adult predators, but adult, invulnerable prey no longer need these cues to avoid predators.

In some cases, adult prey are not just invulnerable to their predators, but they can even kill the juveniles of their predators[11–15,23]. Well-known examples of such role reversals occur when species are involved in reciprocal intraguild predation, *i.e.* predators that compete for food but of which adults also consume each other's juveniles[13–14,24–25]. However, role reversals are also observed in systems that are viewed as classical predator-prey systems[12,15–16]. For example, Saito[11] showed that adults of the spider mite prey *Schizotetranychus celarius* attack and kill juvenile stages of their predator, the phytoseiid mite *Typhlodromus bambusae*, and Aoki and co-authors[16] observed first-instar larvae of the sugar-cane woolly aphid, *Ceratovacuna lanigera*, attacking and piercing predator eggs with their horns. In some cases, adults kill the juveniles of the other species but do not consume them, suggesting that the killing serves to reduce future predation risk or competition[11,14,16].

We studied the effect of exposure of juvenile prey to predators on the antipredator behaviour of surviving prey later in life, after role reversal had occurred. We exposed groups of prey during their entire juvenile development to adult predators and then tested whether the prey that survived this exposure until adulthood showed higher predation rates on the juveniles of these predators than prey that had not been exposed.

There is an obvious danger with such an experimental set-up in that predation during the exposure to predators might act selectively on the juvenile prey, with some individuals being more susceptible to predation than others[26]. This variation in the behaviour of the juvenile prey may be part of a behavioural syndrome[27], where lower susceptibility is correlated with adult behaviour later in life. For example, if boldness and aggressiveness in juvenile prey would result in lower predation risk, predation would weed out the least bold and the least aggressive juvenile prey, and the remainder of the prey population would consist of individuals that may also act relatively more aggressive towards juvenile predators. We therefore controlled for selective predation by mimicking it. We removed data of individuals with the most extreme behaviour from the control group (i.e. not exposed to

predators) to a number equivalent to the number of individuals killed in the group that was exposed to predators. If the difference in behaviour between the control group and the exposed group would be due to selective predation, there would be no difference in behaviour between the exposed group and the reduced control group. A significant difference in behaviour of the exposed group compared to the reduced control group would point at a change in behaviour as a result of the exposure to predators.

We used this method to study three predatory mite species: *Iphiseius degenerans* (Berlese), *Neoseiulus cucumeris* (Oudemans) and *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae). These mites are small (adults $<$ 1 mm), blind predators of other mites and small insects, but they also feed on food sources derived from plants such as pollen. The species studied here co-occur in the Mediterranean area[28], and feed and reproduce on thrips larvae and pollen as a common food source[29–31]. Moreover, adults and juveniles of *I. degenerans* and *N. cucumeris* feed on the juvenile stages of the other species, with larger stages attacking smaller ones, even when alternative food (such as pollen) is available[24,32]. Thus, they are reciprocal intraguild predators and the designation "prey" and "predator" depends on the situation. Here, *I. degenerans* is consistently referred to as "prey" and *N. cucumeris* as "predator" to facilitate interpretation. We show that exposure of juvenile prey to adult predators during their entire ontogeny affects the prey's behaviour towards juvenile predators after they turned adult.

Results

Effect of exposure of juvenile prey to predators. Groups of 20 juvenile prey eggs were exposed from egg to adulthood to 5 adult predators in the presence of food (pollen). In this way, prey experienced the threat of predation during their entire development. The survival until adulthood of these exposed prey was 26.6% lower than that of prey that developed without predators (control), showing that the predation risk was substantial (mean survival $\pm$ s.e.m.: 55.9% $\pm$ 2.60 and 82.5% $\pm$ 2.14 respectively, generalized linear model (GLM) with quasi-binomial errors, $P < 0.0001$, $n = 22$ for each treatment). Males and females of the prey had similar egg-adult survival rates.

We subsequently randomly selected one adult female prey from each group of surviving prey and tested the predation behaviour of these prey individuals towards juvenile predators. Prey that had been exposed to adult predators killed juvenile predators at a significantly higher rate than prey from the control group, which had not been exposed (Fig. 1A; GLM, $X^2 = 14.7$, df $= 1$, $P < 0.001$, n $= 22$ each for treatment and control).

To verify whether this increased killing rate was specifically aimed at the predator species to which the prey had been exposed, we exposed juvenile prey as above, and offered larvae of another predatory mite species, *Amblyseius swirskii*, to a randomly chosen surviving adult female prey. These larvae are of similar size and mobility as those of *N. cucumeris*. Adult prey that had been exposed to adult predators during juvenile development killed juveniles of *A. swirskii* at a similar rate as adult prey from the control group (Fig. 1B; GLM, $X^2 = 0.4$, df $= 1$, $P = 0.529$, $n = 22$ each for treatment and control).

One explanation for the increased attack rate by experienced prey is that the prey that had been exposed to predators were hungrier than unexposed prey and therefore killed more juvenile predators. Although there was ample food (pollen) available during the exposure to predators, prey may have displayed antipredator behaviour, resulting in less time spent feeding. Hence, they may have ingested less food before the start of the experiment than the control group and this could have caused them to feed more on juvenile predators. However, the prey that had been exposed to adult predators and then offered juvenile *A. swirskii* should have been starved to a similar extent as those that had been exposed and offered juvenile predators, but they did not show a higher predation rate (Fig. 1B). Moreover, oviposition rates and satiation levels are closely correlated in predatory mites[33], and there was no difference whatsoever in the oviposition rates of exposed vs. unexposed prey (Kruskal-Wallis test with 1 d.f., at 3 h: $X^2 = 0.41$, $P = 0.52$, at 5 h: $X^2 = 0.089$, $P = 0.77$, at 24 h: $X^2 = 0$, $P = 1$; no eggs were produced at 1 h). All females except for one in the control group oviposited. Furthermore, the increased killing of juvenile predators cannot have served to complement the diet of the adult prey, because this would equally hold for the control group, and they killed juvenile predators at a lower rate. It is therefore concluded that the difference in predation of juvenile predators (Fig. 1A) was not caused by differences in satiation levels of the adult prey, but by other changes in the motivational state of the prey.

As argued in the introduction, the difference in behaviour between exposed and unexposed prey (Fig. 1A) could have been a consequence of selective predation during the exposure. To mimic

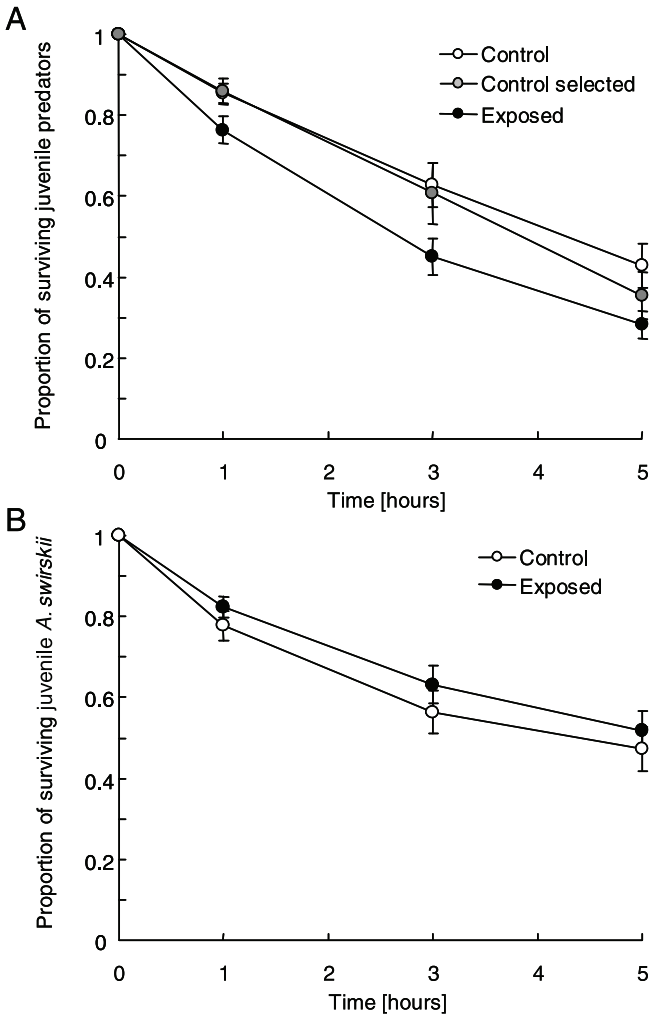

Figure 1 | Predation of juvenile predators by adult prey that were exposed to adult predators during their development. Shown is survivorship of groups of ten (A) juvenile predators (*N. cucumeris*) and (B) juveniles of another predator species (*A. swirskii*) when exposed to adult female prey (*I. degenerans*) that had either been exposed to adult predators (*N. cucumeris*) when juvenile (Exposed, black symbols, n = 22) or not (Control, white symbols, n = 22). Control selected (grey symbols) concerns a subset of the Control group consisting of the 15 individuals that killed most juvenile predators. Shown are the Kaplan-Meier estimates and the standard errors based on the Greenwood formula for the variance (Crawley 2007). Mortality of juvenile predators of both species was due to killing by the adult prey (*I. degenerans*). As almost all juvenile predators of both species were killed within 24 h, survival after 24 h is not shown in this graph, yet these data were included in the statistical analysis.

selective predation, we removed data of those individuals from the control group that killed the fewest juvenile predators when they had become adult, thus reducing the difference between the exposed group and the control group. Seven individuals were removed, corresponding to the 26.6% predation that occurred in the group that was exposed (see above). Subsequently, we compared the predation imposed by the reduced control group (now consisting of the 15 individuals that killed most juvenile predators) with that of the entire group of exposed prey. The difference in predation rate between the control and exposed prey was still significant (Fig. 1A, control selected vs. exposed: GLM, $X^2 = 4.6$, df = 1, $P = 0.0318$). We therefore conclude that even if selective predation during exposure to predators did occur, it was not the primary factor that caused the difference between exposed and control group. Hence, the increased attack of juvenile predators by prey that had been exposed to predators during their ontogeny was for a large part due to changes in the behaviour of individual prey that survived this exposure.

Exposure of adult prey to adult predators. In the experiment described above, prey were exposed to adult predators until they had reached adulthood, hence, they were exposed to adult predators in their early adult phase, albeit during a short period. To determine whether the exposure of adult prey was sufficient to induce the increased predation as observed above (Fig. 1A), adult prey were exposed to adult predators during two days and were subsequently tested for predation on juvenile predators as above. After this exposure, the adult prey killed juvenile prey at a similar rate as adult prey that had not been exposed (Fig. 2; GLM: $X^2 = 0.2$, df = 1, $P = 0.635$, $n = 14$ for each treatment).

Killed juvenile predators deter adult predators. Although adult prey feed on juvenile predators, this does not increase their survival or oviposition when pollen is present as alternative food[24–25,32], as was the case in our experiments. The question thus remains why adult prey kill more juvenile predators after having been exposed to adult predators during their juvenile period. One explanation is that the killing of juvenile predators deters adult predators, thus reducing the risk of predation of the offspring of the adult prey[15,23]. We therefore measured predation on juvenile prey by adult predators in the presence or absence of dead bodies from killed juvenile predators. Significantly fewer juvenile prey were killed in the presence of cues of killed juvenile predators than in the absence of such remains (Fig. 3; linear mixed effects model (LMER), $X^2 = 9.22$, df = 1, $P = 0.0024$, $n = 13$ for each treatment). This shows that the killing of juvenile predators by adult prey can indeed result in increased survival of juvenile prey. Adult predators also laid a significantly smaller proportion of their eggs near the remains of killed juvenile conspecifics (Fig. 3; LMER, $X^2 = 5.39$, df = 1, $P = 0.020$), but the total number of eggs (on both sides of the arena) did not differ significantly between treatments (control: 0.92 ± 0.21; with killed juvenile predators: 1.38 ± 0.27, GLM, $X^2 = 1.2$, d.f. = 1, $P = 0.272$).

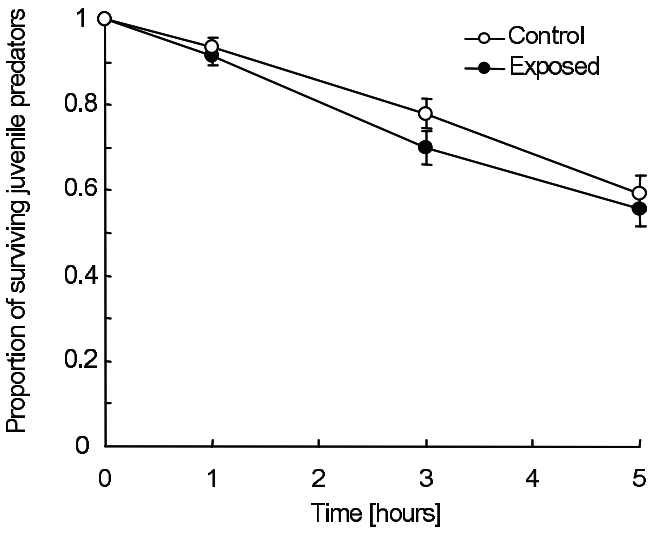

Figure 2 | Predation of juvenile predators by adult prey that were exposed to adult predators during the first days of the adult prey stage. Shown is survivorship of groups of ten juvenile predators exposed to adult female prey that had either been exposed to adult predators when adult (Exposed, closed symbols, n = 14) or not (Control, open symbols, n = 14). See legend to Fig. 1 for further explanation.

Discussion

Our experiments showed that prey that were exposed to adult predators when juvenile, developed into adults that killed more juvenile predators per unit of time than non-exposed prey. This was most likely caused by changes in the behaviour of the individual prey, not by selective predation during the exposure to predators. This shows that prey can tune adult antipredator behaviour based on juvenile experience after an ecological role reversal. We furthermore showed that the increased attack of juvenile predators deterred adult predators and consequently reduced predation risk of juvenile prey.

We furthermore showed that selective predation during the exposure of juvenile prey to adult predators is an unlikely explanation for the increased attack rate. We mimicked selective predation by removing the prey that killed the fewest juvenile predators from the control group. Assuming a syndrome of correlated behaviours[27], the only explanation for our results would have been that bold and aggressive juvenile prey would run a lower predation risk, whereas bold, aggressive adult prey would kill more juvenile predators. Hence, selective predation would then favour bold, aggressive juvenile prey. However, the opposite could also be argued, i.e. that bold, aggressive juvenile prey would run a higher predation risk. However, individuals that survived exposure to adult predators were more ferocious than individuals that had not been exposed, showing that this latter scenario is unlikely to have occurred.

Commonly used methods to avoid the problem of selective predation in the study of antipredator behaviour are to expose the prey to cues of predators or to predators that are in some way restrained so as not to be able to kill prey, for example by being placed in cages or by their mouthparts being manipulated to prevent them from killing prey[34]. Although these methods have often been used[4,22,35–36], they

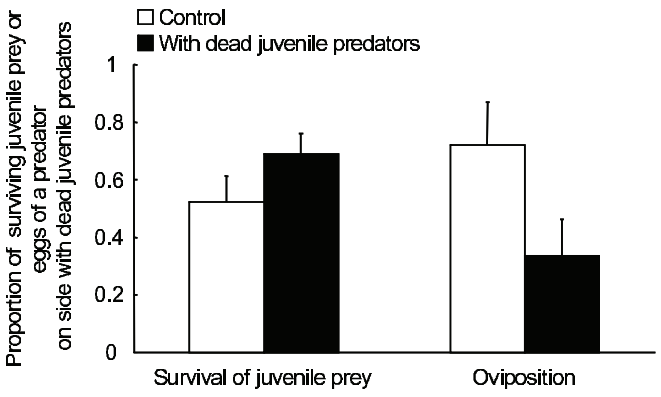

Figure 3 | Killing juvenile predators protects prey offspring. Shown is survival of juvenile prey and eggs laid by an adult female predator on an arena with dead juvenile predators (black, n = 13) or without (Control, white, n = 13). Shown is the fraction (mean + s.e.m.) of juvenile prey surviving on the entire arena and the fraction (mean + s.e.m.) of eggs laid on the patch on which the juvenile prey were released. **: $P < 0.01$; *: $P < 0.05$.

may not be fully adequate for long-term experiments. It is common knowledge that scarecrows are not very efficient in keeping crows at bay because the birds rapidly learn that they have nothing to fear from these straw men. Such "scarecrow effects" are likely to occur in prey that are exposed to restrained predators or predator cues for prolonged periods, without there being actual predation risk. Experience has been shown to reinforce antipredator behaviour[18–22], and the behaviour of prey exposed to predator cues without experiencing predation risk would not be reinforced. Hence, prey will gradually ignore restrained predators or cues of predators because they are no longer associated with predation risk. Another argument against prolonged exposure of prey to restrained predators is that predators that are not capable of killing prey are likely to change their foraging behaviour as a consequence. The predators' hunger level increases during long-term experiments, and this may result in them (unsuccessfully) attacking many more prey than unrestrained predators would normally attack, because the latter would become satiated after the consumption of some prey. Alternatively, restrained predators may perceive that their attacks are futile or they may be weakened because of lack of food, leading to fewer and less severe attacks. Taken together, it is highly unlikely that restrained predators or predator cues would have the same effect on prey behaviour as real predation risk. We therefore suggest that mimicking selective predation as done here is better than exposing prey to constrained predators or cues associated with predators, particularly in experiments that last long enough for the prey to adapt to such cues (the scarecrow effect).

Our results show evidence for recognition of species by the prey: adult prey killed more juvenile predators only when predator juveniles were of the same species as to which the prey had been exposed in the juvenile phase (Fig. 1). This could be caused by a preference of adult prey for juveniles of the predator species to which they had been exposed. However, adult prey from the control group killed equal amounts of larvae of both *N. cucumeris* and *A. swirskii*, showing that they had no preference for either species of juvenile predators when pollen was present (cf. survival of juveniles of both predator species in the presence of adult prey of the control group, Fig. 1A,B: $X^2 = 2.9$. d.f. $= 1$, $P = 0.089$). Notice that a preference for larvae of *N. cucumeris* would not explain the main results, i.e. the increased predation rate of prey that were previously exposed to predators relative to the control group (Figure 1A).

Our results thus imply that adult female prey recognize the juveniles of their childhood foe, whereas they were only exposed to adult females and eggs of this predator when young. We attempted to further test this by exposing juvenile prey to another predator, i.e. *A. swirskii*, but none of the juvenile prey survived exposure to this voracious predator, hence, the test could not be completed. Meanwhile, we do not know how the prey recognized the juveniles of the predator species to which they had been exposed. In general, prey are known to perceive predation risk via excretion and secretion products of predators[37–38], and by alarm pheromones produced by conspecific prey[5,39–40]. However, the behavioural changes observed in our experiments cannot have been a response to such cues because these were absent when the predation by adult prey was tested. Possibly, adults and juveniles of the predator carry the same cues (perhaps cuticular hydrocarbons), which would then facilitate species recognition. Yet, the response to these cues would then be dependent on the developmental stage of the prey: juvenile prey that perceive cues of adult predators should try to escape, whereas adults that perceive cues of juvenile predators counterattack. Perhaps they learn the association between predator-specific cues and the risk of predation when exposed to the adult predators and this learning experience carries over to later in life[41], when they recognize the juveniles of their childhood enemies by the same cues. The fact that adult prey that had experienced adult predators, but no predation, did not kill more juvenile predators (Fig. 2) suggests that the association of predator cues with predation risk is indeed essential to induce changes of behaviour in adult prey. It is still an open question why the prey do not simply always kill juvenile predators at the same rate, but instead need experience to increase their killing rate. We suspect that this is related to the costs associated with this antipredator behaviour. Perhaps the adult prey risk being injured when attacking juveniles of other predatory mite species[42–43], and they should therefore only attack juveniles of the species that pose a serious threat to their offspring.

One question that remains is how our results translate to predator-prey systems in the real world. In our experiments, prey and predators were confined to small arenas in order to study their behaviour. As a consequence, juvenile predators and prey could not escape from attacks. Notably, the mortality of juvenile predators of all species was high after 24 h of exposure to adult prey (see legend to Figure 1). We expect that some of these juveniles would escape from the attacking adult prey under more natural conditions. Likewise, adult prey may prefer to settle on patches where adult predators do not kill juvenile prey. Hence, the behaviour we describe here will probably give rise to distributions of predators and prey that depend on the stage of the individuals of the species present on any given patch as well as on the experience of these individuals[44]. This will result in spatial separation of predators and prey: prey can drive away predators by killing their young, but at the same time will avoid settling in patches occupied by predators[5,45–46]. Such spatial separation will reduce the interaction strength between predators and prey[47].

The species studied here are engaged in intraguild predation, an interaction in which two species compete for resources and one of the species (the intraguild predator) attacks and feeds on the other (the intraguild prey)[13]. Classic theory, derived from simple Lotka–Volterra models on well-mixed populations, predicts limited possibilities for coexistence of such species[13,48–49]. Usually, intraguild prey either exclude intraguild predators through competition for resources or intraguild predators exclude intraguild prey through predation[13,48–49]. Indeed, several experiments have shown extinction of populations of one of the two species engaged in intraguild predation[24,50–51]. Persistence of intraguild prey and intraguild predators is, however, possible at larger spatial scales, where populations of each species can occupy patches of the shared resource[52]. However, patches occupied by the intraguild prey will then still be vulnerable to invasion by the intraguild predator. By attacking juvenile intraguild predators, intraguild prey decrease the success of such invasions[25], hence, the order of invasion of patches will determine which species will persist[53], thus increasing persistence of populations at a metapopulation scale. We expect that the increased counterattacks of juvenile predators by adult prey that have experienced predation risk will further reduce the invasion success of intraguild predators, thus ensuring increased persistence of local populations of the intraguild prey through antipredator behaviour[54].

Another question that begs and answer is why adult invulnerable prey would kill harmless juvenile predators. Other experiments have shown that such killing and consumption of juvenile predators did not directly increase the survival or oviposition of the adult prey individuals[24]. However, the behaviour may serve as a form of maternal care[11]: we found here that survival of juvenile prey was increased in the presence of killed juvenile predators. Hence, adult prey can deter predators by killing predator offspring[15], thus creating a place with lower predation risk for their own offspring. Although interspecific infanticide has been reported for several species[12–14], its function is often unclear (but see Saito[11]). Palomares and Caro[14], for example, reported that many mammalian carnivores kill young of other species, sometimes without feeding on them. There is also at least one example of adult African buffalo killing lion cubs[55], but an explanation for this behaviour, which is risky for the buffaloes, was, to our knowledge, never proposed. We suggest that such behaviour

serves to expel predators, thus increasing the survival probability of the vulnerable offspring of the adult prey. We furthermore suggest that experience at a vulnerable stage serves to fine-tune such risky antipredator behaviour when prey may potentially interact with various species of predators. We expect that the killing of juvenile predators by adult prey occurs more frequently than thought thus far, particularly in prey species that are vulnerable when young and invulnerable when adult, and when these prey have several potential predators, to which they have to fine-tune antipredator behaviour.

Methods

The origin of the cultures of *I. degenerans* and *N. cucumeris* and the methods to rear these species are described elsewhere[30]. In short, they were reared on plastic arenas, placed on top of a sponge in a water-containing tray. The edges of the arenas were covered with tissue paper that extended to the water in the tray. In this way, the tissue served both as a barrier and as a water source. *Iphiseius degenerans* cultures were fed twice per week with birch pollen (*Betula pubescens* Ehrh.), *N. cucumeris* was fed *Typha* sp. pollen. *Amblyseius swirskii* was collected in Israel (location Revadim) in 1997[56], and was reared using the same method as *N. cucumeris*. All mite species were maintained without any animal prey as a food source. Conditions of rearing and experiments were 25±1°C, 16/08 hours L/D, and 60±5% RH.

Exposure of juvenile prey to adult predators. Groups of 20 prey eggs (*I. degenerans*) and five adult predators (*N. cucumeris*,10–14 days post eclosion) were placed on plastic arenas (5 × 5 cm) on top of a sponge in a water-containing tray. Ample amounts of *Typha* sp. pollen were deposited in the centre of the arena and served as food, allowing both species to feed freely on it, without competition for food. Pollen is an excellent food source for the prey and predators, the egg production of both prey and predators on pollen is equal to that on a mixed diet of pollen and juveniles of the other species[24–25]. Although the adult predators are small and the juvenile prey that emerge from these eggs are considerably smaller, they are both mobile and can easily cross the entire arena within ten minutes. Hence, during the development to adulthood, the juvenile prey will inevitably have contacted adult predators and their cues as well as killed conspecifics. To prevent escape, the edges of the arena were covered with wet paper tissue as described above. All eggs of predators were removed with a fine paint brush every other day. Thus, juvenile and young adults of the prey were exposed to adult females (hence also eggs) of the predators, but not to larvae or nymphs. The same number of prey eggs were placed on similar arenas, yet without adult predators, and served as a control. We repeated the above procedures 22 times per treatment. The number of prey surviving to adulthood was assessed 10 days after their introduction to the arena.

From each arena, one surviving female prey was randomly selected and was placed on a plastic disc (3.2 cm diameter) on water-saturated cotton wool in a plastic container. Ten juvenile predators were also placed on the disc together with an ample supply of pollen. Predator larvae of one day old post-hatching were randomly selected for the two treatments. The water barrier confined the mites on the arena. The number of surviving juvenile predators was assessed after 1, 3, 5 and 24 h. Because unmated females feed less than mated females, the eggs produced by the adult prey were also counted at the same time points as above to verify that the adult females were mated. One female from the control group that did not oviposit was excluded from further analysis. Background mortality of juvenile predators in the presence of pollen was extremely low and cannibalism among the juvenile predators does not occur under these conditions[57], hence, we attributed all mortality of juvenile predators to predation by the adult prey. Predation was verified by searching for the remains of killed juvenile predators on the arena. The predation rates by 22 adult prey that had been exposed to adult predators as juvenile and 22 unexposed adult prey were compared.

To test whether an adult prey discriminated between juveniles of the predator species to which it had been exposed and a novel predator species, other groups of juvenile prey were exposed to adult predators as above, but surviving adults were subsequently offered juveniles of *A. swirskii* instead of juveniles of *N. cucumeris*. *Amblyseius swirskii* are also predators of juveniles of other predatory mites, including *I. degenerans* (A. Janssen, pers. obs.). Here too, 22 adult prey that had been exposed and 22 unexposed adult prey were tested, and data were collected as above.

Data on the survival of prey until adulthood were compared using a generalized linear model with quasi-binomial error distributions to correct for overdispersion (GLM in R[58–59]). Using survival analysis (Kaplan-Meier survivorship of the survival library of R[59–60]), the time course of mortalities due to predation by adult female prey that had been exposed to adult female predators during their development or not were compared. Oviposition of adult prey was compared with a Kruskal-Wallis test. All tests were two-tailed and we used an alpha level of 0.05.

Exposure of adult prey to adult predators. Ten adult prey (males and females of 8 days post eclosion) and five adult female predators (18–22 days old) were placed on a plastic arena as used for exposure of juvenile prey (see above). A similar group of adult prey were placed on similar arenas, but without adult predators, and served as a control. Ample amounts of *Typha* sp. pollen were added at the centre of the arena as a food source for both species to avoid competition for food.

From each arena, one female prey was randomly selected after 2 days and was placed in a plastic Petri dish (3.2 cm diameter; and 0.9 cm high) together with ten juvenile predators of 1 day old and a piece of water-saturated cotton wool that served as a water source. The Petri dishes were larger than the arenas described above, in an attempt to decrease the encounter rate of the adult prey with the juvenile predators. This was expected to result in lower predation rates, and higher survival of juvenile predators after 24 h, so it would be possible to see differences between the treatments even after 24 h (nearly all juvenile predators were preyed after 24 h in the previous experiment). However, this did not have the desired effect; most larvae were killed after 24 h anyway. As above, the number of surviving juvenile predators was counted 1, 3, 5 and 24 hours after the introduction. The experiment was repeated 14 times. The predation by adult prey that had been exposed to adult predators was compared with that of the unexposed control group using survival analysis as above.

Killed juvenile predators deter adult predators. To test whether the killing of juvenile predators affected predation risk of the offspring, plastic arenas consisting of two patches were used (diam. 36 mm), connected by a small strip of plastic (6 cm long, c. 3 mm wide). Ten juvenile predators were released on one of the two patches and were immediately killed by piercing them with a fine needle to simulate predation. Subsequently, we released ten juvenile prey on the same patch, and an adult female predator was released at the centre of the bridge, from where she could walk to either of the two patches. We used adult female predators that had no experience with prey. The other patch received no juvenile predators and served as an alternative to the treated patch. Each patch contained ample *Typha* pollen, hence, there was no competition for food. As controls, we used similar arenas on which ten juvenile prey and one adult female predator were released as above, but no juvenile predators were added and killed. Hence, the only difference between the experimental and control arenas was the presence of killed juvenile predators. We scored the numbers of surviving juvenile prey on the entire arena (both patches) and the number and position of adult female predator eggs 24 h later. The proportion of juvenile prey on the arena side with the remains of killed juvenile predators (3.31 ± 0.51) was not significantly higher than that on the other side (2.62 ± 0.40, binomial test, $P = 0.18$), showing that the prey larvae could and did move to the other side. The experiments of both control and treatment were repeated 13 times in total, with two blocks of 6 and 7 replicates respectively. Differences in survival of juvenile prey and predator oviposition on treated and control patches were analyzed with a linear mixed effects model (lmer of the lme4 library of R[58–59]) with a quasi-binomial error distribution to correct for overdispersion and repetition as a random factor. Replicates in which the female predator did not oviposit were excluded from the analysis of oviposition (4 cases of the control and 3 of the treatment). Differences in total oviposition (on both patches) were analysed with a generalized linear model with a Poisson error distribution. The distribution of surviving juvenile prey was tested with a binomial test.

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YC, MI and AJ designed and performed the experiments, YC, AJ and MWS wrote the ms.

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