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DOI

[10.1111/1365-2435.12439](https://doi.org/10.1111/1365-2435.12439)

Publication date

2015

Document Version

Final published version

Published in

Functional Ecology

License

Article 25fa Dutch Copyright Act

[Link to publication](#)

Citation for published version (APA):

Vieira Marques, R., Almeida Sarmiento, R., Lemos, F., Pedro-Neto, M., Sabelis, M. W., Venzon, M., Pallini, A., & Janssen, A. (2015). Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. *Functional Ecology*, 29(10), 1317-1324. <https://doi.org/10.1111/1365-2435.12439>

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Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost

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Summary

1. Mixing of prey that differ in nutrient content or toxic compounds (dietary mixing) may allow synovigenic predatory arthropods to balance their diet or dilute toxins of different prey items to maximize performance: dietary mixing may therefore explain the prevalence of polyphagy in this functional group.
2. Several predatory arthropods can redress nutritional imbalances in their diet by actively mixing different diets, based on experiments with artificial diets or with prey that were manipulated to contain different nutrients.
3. Evidence is also accumulating that predator species perform better on a mixed diet of several different prey species, but evidence that they actively forage for different prey species is scarce.
4. Thus, evidence that predators actively forage for a mixed diet consisting of natural, non-manipulated prey to increase their performance is still rare. Here, we investigate whether arthropod predators invest in achieving an optimal diet by active mixing of different prey species or by simply eating what they encounter.
5. Females of two species of plant-inhabiting predatory mites produced significantly more eggs when feeding on a prey mixture of co-occurring phytophagous mites than when feeding on either of the two prey species separately.
6. When the two prey species were offered on two separate, connected patches at some distance from each other, predators commuted significantly more between the two patches to obtain a mixed diet and had a significantly increased egg production than predators that were offered a single prey species on two patches.
7. Thus, predators actively commuted between patches to balance their diet. We propose that active dietary mixing can have synergistic effects on predator fitness and has the potential to explain polyphagy in this functional group.

Key-words: balanced diet, *Euseius concordis*, foraging behaviour, *Iphiseiodes zuluagai*, nutritional ecology, phytoseiids, *Polyphagotarsonemus latus*, prey quality, *Tetranychus bastosi*

Introduction

Predatory arthropods typically consume many more than one prey species (Sabelis 1992), referred to as polyphagy.

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[†]In memory of our co-author, teacher, colleague and dear friend Maurice Sabelis, who sadly did not live to see the last version of this paper.

Polyphagy prevails in this functional group and contrasts sharply with other functional groups, such as herbivorous arthropods, where specialization on a single host species or genus (monophagy, oligophagy) prevails (Jaenike 1990). For a long time, predatory arthropods were assumed to optimize the rate of prey capture or energy intake per time spent on a prey (Charnov 1976; Stephens & Krebs 1986), rather than selecting prey according to nutrient

composition (but see Belovsky 1978; Greenstone 1979; Rapport 1980; Tinbergen 1981; Bilde & Toft 1994). Over the last decades, however, several alternative hypotheses have been put forward. One explanation for consuming mixed diets is the toxin-dilution hypothesis (Freeland & Janzen 1974), where foraging animals need to mix their diet to dilute the toxins of any particular diet type (Toft & Wise 1999). The other hypothesis is that predatory arthropods need to redress specific nutritional imbalances by feeding on prey species containing different amounts of nutrients (Mayntz *et al.* 2005).

Originally, diet mixing or self-selection was predominantly studied in generalist herbivores (Waldbauer & Friedman 1991; Bernays *et al.* 1994). Herbivorous arthropods are known to forage selectively for protein and carbohydrates to redress specific nutritional imbalances (Raubenheimer & Simpson 1997). They may select among foods of different nutritional composition by eating more of a host plant if it is richer in nutrients that are in short supply, or specifically extracting nutrients from a single host (Waldbauer & Friedman 1991; Simpson & Raubenheimer 1995; Raubenheimer & Simpson 1997, 2003; Simpson *et al.* 2004; Jones & Flynn 2005; Behmer 2009). More evidence for diet mixing in herbivores, omnivores and predators has surfaced during the last decades (Lefcheck *et al.* 2013). As an early example of diet mixing in predators, Bilde & Toft (1994) showed that carabids produce more eggs on a diet consisting of a mixture of dead aphids, fruit flies and earthworms than on any of the single diets, and Toft & Wise (1999) demonstrated positive effects of one mixed diet on some, but not all, aspects of the life history of a spider. Furthermore, beetles, spiders and ants were found to forage selectively for protein or lipids, depending on the previous diet (Mayntz *et al.* 2005; Dussutour & Simpson 2009), and using a geometric framework for nutrition (Simpson & Raubenheimer 1995), Jensen *et al.* (2012) showed that predatory beetles regulate nutrient intake such that their numbers of eggs were maximized.

Both the balanced diet hypothesis and the toxin-dilution hypothesis predict that animals should have higher fitness on a mixed diet than on single-prey diets, and there is mixed evidence for this. A recent meta-analysis shows that animals on mixed diets had a significantly higher fitness than the average of animals feeding on single diets (Lefcheck *et al.* 2013). However, in more than half of the cases reviewed, the best single diet was as good as or better than the mixed diet (Lefcheck *et al.* 2013). The review found little support for the toxin-dilution hypothesis and showed that the positive effects of diet mixing were stronger for generalist herbivores than for other trophic levels.

The experimental evidence of diet mixing in predators can be roughly divided into two categories. On the one hand, there are several elegant experimental studies showing that predators actively redress nutrient imbalances in their diet (Mayntz *et al.* 2005; Raubenheimer *et al.* 2007; Dussutour & Simpson 2009; Jensen *et al.* 2012); however, most of these studies did not use natural prey species, but

(semi-)artificial diets or prey that were specifically reared to contain different amounts of nutrients. On the other hand, several studies show that predators feeding on a mixture of prey perform better (Bilde & Toft 1994; Toft & Wise 1999; Messelink *et al.* 2008; Lefcheck *et al.* 2013), but showing that predators actively forage for a mixed diet is difficult with live prey. As soon as live prey are used, non-random feeding may be caused by prey attributes such as size, activity, crypsis and antipredator behaviour as well as by the predator's choice. Hence, it becomes difficult to ascertain that non-random feeding is caused by active decision-making on the part of the predator. In conclusion, evidence is scarce that predators do actively forage for a mixed diet consisting of several types of *natural* prey, which is the topic we address here.

We studied the reproduction and foraging behaviour of two predatory mite species that feed on two species of prey mites. Many species of predatory mites feed on several prey types and plant-provided foods such as pollen (Croft, Blackwood & McMurtry 2004), and some of them benefit from a mixed diet (Messelink *et al.* 2008). Here, we show that two species of predatory mites have a higher oviposition rate on a mixed diet of two prey species and that the predators actively forage for prey on different patches in order to obtain a mixed diet.

Materials and methods

THE EXPERIMENTAL SYSTEM

We studied two species of predatory mites belonging to the family Phytoseiidae, *Iphiseiodes zuluagai* Denmark & Muma and *Euseius concordis* Chant. Predatory mites are small (<0.5 mm) and blind and feed by inserting their mouthparts into a prey and sucking out (part of) the content. Female predatory mites produce one to few eggs per day and need to feed between ovipositions. They are very efficient at converting food into eggs (Sabelis 1981), hence are ideally suited to study the effects of diets on reproductive performance. As prey, we used the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae). The broad mite is a small mite (maximum 0.2 mm long), which is a pest of various crops and ornamentals (Gerson 1992). Its eggs are flat, elliptical in shape, with the largest diameter being 0.08 mm. The other prey species, *T. bastosi*, attacks various crops and weeds (Sarmiento *et al.* 2011; Cruz *et al.* 2013). Its adult females are the largest stage, and they are c. 0.5 mm long, whereas the eggs are spherical with a diameter of c. 0.13 mm. These herbivorous mites and predators co-occur in plantations of the bio-diesel plant *Jatropha curcas* L. in Brazil, where the pests cause severe yield reductions (Sarmiento *et al.* 2011; Pedro-Neto *et al.* 2013). The predators are currently evaluated for their capacity to control the pests (Sarmiento *et al.* 2011).

PLANT MATERIAL AND MITE REARING

Stock cultures of both predatory mites were started with mites collected from *J. curcas* plants in Gurupi, state of Tocantins, Brazil (11°45'47"S, 49°02'57"W). The predators were reared in arenas consisting of 6-cm-diameter flexible plastic discs floating on distilled water. Castor bean pollen (*Ricinus communis* L.) was added on a daily basis as food for the mites (Reis & Alves 1997). Stock

colonies of the phytophagous mites *P. latus* and *T. bastosi* were started with mites collected from infested native *J. curcas* plants and subsequently transferred to uninfested plants. Clean *J. curcas* plants were cultivated in a greenhouse, where seedlings (15 cm) were individually transplanted into plastic pots (500 mL) containing a mixture of soil and manure in a 3 : 1 ratio. Both predatory and spider mites were maintained inside a climate-controlled room (28 ± 2 °C, 65–70% R.H. and 12 h L/12 h D photoperiod). The experiments were carried out under similar conditions.

PREDATION AND OVIPOSITION ON SINGLE- AND MIXED-PREY DIETS ON ONE PATCH PER ARENA

Two-day-old mated adult females, the most voracious phase in the life of the predators, were tested each on a separate *J. curcas* leaf disc (diameter 3 cm), where they were offered either prey of a single species or a mixture of two species. For each prey species separately, 30 eggs or 30 larvae of one of the two prey species were added to this disc. For mixtures of eggs or larvae of the two prey species, 15 eggs or 15 larvae of each species were offered together. Each treatment was replicated 10 times. Predation was recorded as the number of eggs or larvae consumed during 48 h; oviposition was assessed simultaneously. Consumed individuals were replaced once per 24 h. Accidentally, data on oviposition of *E. concordis* when offered single stages of *T. bastosi* were not collected for five replicates.

PREDATION AND OVIPOSITION ON SINGLE- AND MIXED-PREY DIETS IN AN ARENA WITH TWO CONNECTED PATCHES

Adult females of the predators were offered the two prey species on separate patches connected with a small plastic bridge. Hence, in order to obtain a mixed diet, the predators had to commute between the two patches by crossing the bridge. We used only eggs of the two prey to be able to keep the two prey species spatially separated, which would be impossible with mobile prey stages.

Each arena consisted of two Petri dishes filled with a thin layer of moistened cotton wool (diameter = 9 cm); each dish contained one leaf (diameter *c.* 5 cm), which was connected to the other leaf with a bridge made of plastic (8×0.5 cm). The two patches were *c.* 160 predator lengths apart. Thirty eggs of *P. latus* were transferred to one of the leaves, and the other received thirty eggs of *T. bastosi*. A female predatory mite was placed on the bridge and the patch on which she was found was recorded every 10 min during the first 5 h of the experiment. Control treatments consisted of similar arenas with the same prey species (*P. latus* or *T. bastosi*) on both connected leaves. The experiment was done under conditions as above. Each treatment was replicated 10 times. After 48 h, the number of intact and consumed prey eggs was recorded for each patch and oviposition of the predators was assessed. Consumed prey individuals were replaced after 24 h.

DATA ANALYSIS

Predation rates and square root-transformed oviposition rates were compared using generalized linear models (GLM) with a Gaussian error distribution. The number of times that predators changed patches during the first 5 h was compared among treatments using a GLM with a Poisson error distribution, whereas the proportion of predators that fed on each of the two patches was analysed with a GLM with a binomial error distribution.

When the predators were offered ample amounts of the two prey on one patch, we assumed that the predators would consume a self-selected ratio of the two prey that maximized egg

production (Jensen *et al.* 2012). In order to assess whether predators could obtain the same ratio when they had to commute between two patches to feed on both prey, we used a binomial test to compare this self-selected ratio (proportion of *P. latus* consumed), with the proportion of *P. latus* consumed in the experiment where the two prey were offered on separate patches.

All statistical analyses were done using the statistical software R 3.01 (2013). Treatments were compared through model simplification by combining treatment levels (Crawley 2007). All models were checked by plotting residuals against fitted values and checking normality of the error distribution.

Results

PREDATION AND OVIPOSITION ON SINGLE- AND MIXED-PREY DIETS ON ONE PATCH PER ARENA

The number of eggs consumed differed significantly with prey species (GLM, $F_{1,76} = 40.4$, $P < 0.0001$), predator species ($F_{1,78} = 29.4$, $P < 0.0001$) and with diet (single species vs. mixed, $F_{1,77} = 108.4$, $P < 0.0001$, Fig. 1a). The interaction among these three factors was also significant ($F_{1,72} = 13.2$, $P < 0.001$). When offered prey species separately, both predators consumed significantly more eggs of *P. latus* than of *T. bastosi* (Fig. 1a, single treatments). This is probably caused by the eggs of *P. latus* being smaller than those of *T. bastosi*. On single diets, there was a significant interaction between predator species and prey species ($F_{1,36} = 31.0$, $P < 0.0001$) caused by *I. zuluagai* consuming more eggs of *T. bastosi* than did *E. concordis*, but not more eggs of *P. latus*.

When offered a mixture of eggs of both species, both predators consumed approximately equal numbers of eggs of both species (Fig. 1a). Furthermore, *E. concordis* consumed significantly more eggs of *T. bastosi* when offered together with eggs of *P. latus* than when offered alone, showing that these predators consume more prey when offered a mixed diet.

The rate of oviposition of predators feeding on prey eggs varied significantly with diet (Fig. 1b, *E. concordis*: $F_{2,22} = 10.34$, $P < 0.001$; *I. zuluagai*: $F_{2,27} = 3.77$, $P = 0.036$). Oviposition of both predators was lower on both single diets than on the mixed diet (Fig. 1b).

The number of larvae eaten differed significantly with prey species (GLM, $F_{1,76} = 387.3$, $P < 0.0001$), predator species ($F_{1,78} = 94.0$, $P < 0.0001$) and with diet (single species vs. mixed, $F_{1,77} = 685.2$, $P < 0.0001$, Fig. 1c). The interaction among these three factors was also significant ($F_{1,72} = 71.1$, $P < 0.0001$). Both predators consumed significantly more larvae of *P. latus* than of *T. bastosi* (Fig. 1c), likely caused by *P. latus* larvae being smaller than those of *T. bastosi*. When offered a mixture of larvae of both species, *E. concordis* consumed slightly more larvae of *P. latus* than of *T. bastosi* (Fig. 1c). No significant difference was observed for *I. zuluagai* preying on a mixture.

The rate of oviposition of predators feeding on prey larvae also varied significantly with diet (Fig. 1d, *E. concordis*:

Fig. 1. Predation and oviposition on single- and mixed-prey diets on one patch per arena. (a, c) The average predation (+SE, $N = 10$ for all treatments) of eggs (a) or larvae (c) of two prey species (the spider mite *Tetranychus bastosi*, white bars, and the broad mite *Polyphagotarsonemus latus*, grey bars) by two phytoseiid predators (*Euseius concordis* and *Iphiseiodes zuluagai*) during 48 h. Predators were either offered eggs or larvae of a single species (Single), or a mixture of eggs or larvae of both species (Mixed). Asterisks indicate significant differences in numbers of prey eaten within each treatment (Single or Mixed) for each predator separately: *** $P < 0.0001$; ** $P < 0.01$; ns: not significant. Different letters in columns refer to significant differences in the numbers of eggs eaten per prey species in the single and mixed diets for each predator separately [contrasts after generalized linear models (GLM), $P < 0.05$]. (b, d) The average number of eggs produced by the predatory mites when feeding on the two single diets and on a mixed diet during 48 h. White bars: diet of eggs (b) or larvae (d) of *T. bastosi*, dark grey bars: diet of eggs or larvae of *P. latus*, light grey bars: diet of eggs or larvae of both species (Mixed). Within each predator species, bars with different letters differ significantly (contrasts after GLM, $P < 0.05$).

$F_{2,22} = 17.0$, $P < 0.001$; *I. zuluagai*: $F_{2,27} = 22.8$, $P < 0.0001$). Oviposition of both predators was lower on both single diets than on the mixed diet (Fig. 1d).

PREDATION AND OVIPOSITION ON SINGLE- AND MIXED-PREY DIETS IN AN ARENA WITH TWO CONNECTED PATCHES

During the first 5 h of the experiment, the frequency of changing from one patch to the other when the two patches contained different prey species did not differ significantly from that on sets of patches containing the same prey species (GLM: *E. concordis*: Deviance = 3.85, d.f. = 2,27, $P = 0.146$; *I. zuluagai*: Dev. = 3.66, d.f. = 2,27, $P = 0.16$). The two predator species changed from one patch to the other on average *c.* 0.5 times during this period. However, the proportion of predators that consumed prey on each of the two patches was significantly higher after 48 h when the patches contained different prey

species than when both patches contained the same prey species (Fig. 2a, GLM: *E. concordis*: Dev. = 10.5, d.f. = 2,27, $P = 0.0053$; *I. zuluagai*: Dev. = 13.8, d.f. = 2,27, $P = 0.001$). This clearly shows that all predators moved from patch to patch in arenas with both prey present in order to feed on a mixed diet.

There was no significant effect of predator ($F_{1,78} = 0.06$, $P = 0.80$) or prey species ($F_{1,77} = 0.25$, $P = 0.62$) on predation (Fig. 2b). When offered single and mixed diets, both predators consumed approximately equal numbers of eggs of both species (Fig. 2b). We assumed that predators could easily obtain both prey when offered together on the same patch (Fig. 1a, Mixed), hence fed on the optimal mixture of eggs of the two prey species. A comparison with the diet obtained on the two separate patches (Fig. 2b, Mixed) would then indicate whether the mites also obtained an optimal diet when they had to search for the prey on two different patches. On a mixed diet on a single patch, 47.1% of the eggs consumed by *E. concordis* were of

P. latus (Fig. 1a). When feeding on a prey mixture offered on two patches, one out of 10 predators consumed a significantly lower proportion of *P. latus* than this (25%, binomial test, $P = 0.038$), whereas the diet composition of the other predators did not differ significantly. Overall, 47.8% of the eggs consumed by the predators feeding on a mixed diet on two separate patches were of *P. latus* (Fig. 2b), and this did not differ from expected (binomial test, $P = 0.61$). For *I. zuluagai* feeding on a mixed diet on a single patch, 45.6% of the eggs consumed were of *P. latus* (Fig. 1a). When feeding on eggs of *P. latus* and *T. bastosi* offered on separate patches, 2 out of 10 predators consumed a significantly lower proportion of *P. latus* eggs than expected (23.1% and 14.3%, $P = 0.034$ and $P = 0.015$) and one predator consumed a significantly higher proportion of *P. latus* eggs than expected (76.2%, $P = 0.015$). Overall, the proportion of *P. latus* eggs in the diet of *I. zuluagai* feeding on a mixed diet on two separate patches was 46.8% (Fig. 2b) and did not differ significantly from that of predators feeding on a mixed diet on a single patch (binomial test, $P = 0.65$).

Although the predators would have spent time and energy in moving from patch to patch, the oviposition of the predators was significantly higher in the case where eggs of the two prey species were offered on separate patches than when both patches contained eggs of the same prey species (Fig. 2c, GLM: *E. concordis*: $F_{2,27} = 3.59$, $P = 0.042$; *I. zuluagai*: $F_{2,27} = 12.5$, $P = 0.0001$). This confirms that feeding on a mixed diet results in higher rates of oviposition for both predator species. Furthermore, the oviposition rates of predators that had to commute between patches to obtain a mixed diet (Fig. 2c) did not differ significantly from those of predators that were offered a mixed diet on a single patch (Fig. 1b) (GLM, *E. concordis*: $F_{1,18} = 0.46$, $P = 0.506$; *I. zuluagai*: $F_{1,18} = 2.03$, $P = 0.170$). The oviposition rate of the predators that fed on a diet that differed significantly from that expected (see previous paragraph) was also not notably lower than that of other predators; all of them produced two eggs.

Discussion

We show that the predatory mites *E. concordis* and *I. zuluagai* feed and reproduce on each of two phytophagous prey mites separately, but a mixed diet of the two prey species resulted in a higher egg production rate of both predatory mite species. Most importantly, the predators actively foraged for a mixture of these two diets by commuting from a patch with one prey species to a patch with the other prey species. Hence, these small predatory mites can be added to a growing list of predators that benefit from a mixed diet (Lefcheck *et al.* 2013) and maximize reproductive performance by feeding on a mixed diet (Mayntz *et al.* 2005; Raubenheimer *et al.* 2007; Dussutour & Simpson 2009; Jensen *et al.* 2012). Ours is also one of the few studies that show diet mixing with genuine prey co-occurring in the field, and not with artificial diets or

Fig. 2. Predation and oviposition on single- and mixed-prey diets in an arena with two connected patches. Predators (*Euseius concordis* and *Iphiseiodes zuluagai*) were offered prey eggs on two patches connected with a bridge. Both patches contained either eggs of *Tetranychus bastosi* or eggs of *Polyphagotarsonemus latus* (Single), or one patch contained eggs of *T. bastosi* and the other eggs of *P. latus* (Mixed) (a) The average proportion (+SE) of predators that fed on prey eggs on both patches during 48 h. Per predator species, bars with different letters differ significantly [contrasts after generalized linear models (GLM), $P < 0.05$]. (b) The average numbers (+SE) of eggs consumed per prey species on single and mixed diets. ns: No significant difference in the number of eggs eaten per predator and per prey species in single and mixed diets. Note that the prey densities in this experiment were lower than those in Fig. 1a, so predation rates cannot be compared with the previous experiment. (c) The average numbers (+SE) of eggs produced by the two predators when feeding on eggs of *T. bastosi*, of *P. latus*, or a mixture of eggs (Mixed). Per predator species, bars with different letters differ significantly (contrasts after GLM, $P < 0.05$).

prey that were manipulated to contain different levels of nutrients or toxins.

Possibly, the predators tried to compensate for deficiencies in the single diets by consuming higher amounts of the deficient diet, (Simpson *et al.* 2004). For example, they might have differentially extracted nutrients from a single prey to restore their nutritional balance (Mayntz *et al.* 2005). However, it is impossible to estimate whether predators feeding on a mixed diet consumed more or less food in total than when feeding on a single diet because the two prey species differ in size. The only exception to this is *E. concordis*, which consumed significantly fewer eggs of *T. bastosi* when feeding on a single diet than when feeding on a mixed diet (Fig. 1a). A similar trend, but not significant, was seen for this predator feeding on *T. bastosi* larvae (Fig. 1c). This suggests that a mixed diet of the two prey species is easier to digest than a single diet of eggs of *T. bastosi*. This could be caused by *T. bastosi* being toxic to the predators, which would then suffer less adverse effects of this toxin when it is diluted with other food. We did not find a trend of higher predation rates of this predator on mixed diets when prey were offered on two separate patches (Fig. 2b), which is possibly due to the lower numbers of eggs consumed in general in this experiment (*cf.* Figs 1a and 2b). This, in turn, was likely caused by the lower densities of prey eggs offered in the experiment with two connected patches.

By mixing their diet, the predators presumably increased the intake of multiple nutrients or diluted the toxins contained in the different prey species. Because a single diet of each of the two prey species resulted in a lower predator oviposition rate than a mixed diet, the two prey necessarily need to contain different toxins or have a shortage of different nutrients. It is as yet unknown which toxins are present in both prey species or which nutrients are in short supply in one of the prey species and abundant in the other. It is therefore not possible yet to manipulate the quality of the prey in order to construct a fitness landscape as a function of specific nutrients, as has been done for several other species, using a geometric framework (Simpson & Raubenheimer 1995; Simpson *et al.* 2004, 2006; Mayntz *et al.* 2005; Behmer & Joern 2008; Lee *et al.* 2008; Behmer 2009; Jensen *et al.* 2012). However, it would be possible to construct a similar fitness landscape as a function of the proportion of the two prey species consumed. Thus, the performance of the predators could be assessed with decreasing numbers of either of the two prey species in order to assess the optimum consumption of each prey. The current experiments were done with ample numbers of each of the two prey species; hence, the predators that were offered both prey on the same patch could choose an optimal diet.

Whereas the predators consumed significantly different numbers of the two prey species when feeding on single diets on one patch, they often consumed similar numbers of both prey when feeding on a mixed diet on one patch (Fig. 1a,c). This may have been caused by the predators

trying to obtain a mixed diet that maximized egg production (self-selection), but alternatively, may have been caused by the predators encountering and consuming more of the larger *T. bastosi* than of the small *P. latus*. Moreover, the larvae of the two species may differ in antipredator behaviour. Although the numbers of both prey consumed in a mixed diet on a single patch did not differ significantly in most cases (Fig. 1a,c Mixed), this does not mean that the predators had no preference for either of the two prey species or that they were not foraging for a balanced diet. The reason for this is that the prey are of different sizes and the behaviour of the larvae of the two species is likely to differ; hence, the encounter rates with the prey were most likely not equal. Because *P. latus* is much smaller than *T. bastosi*, the encounter rate with the former may have been much lower than with the latter species; hence, the equal numbers of both prey eaten when offered together on the same patch could point at the predators trying to actively mix their diet, but this is by no means certain.

Likewise, the increased egg production resulting from two prey species being offered together (as compared to each alone) does not necessarily indicate that predators actively forage for this optimal diet. If the prey mixture results from summing the prey densities of single prey species (a so-called additive design), there is simply more prey, and ingestion of more food can cause higher egg production. This cannot be solved by replacing prey items of one type with prey items of another type (a so-called replacement design), because prey attributes such as vulnerability, size, crypsis and antipredator behaviour may depend on the two prey species being presented separately and together and so will affect egg production. Moreover, the amount of food available in prey items of different species may differ due to differences in the ingestible food content. Thus, both with an additive or a replacement type of experiment (Connolly 1988; Snaydon 1991; Jolliffe 2000), it remains notoriously difficult to interpret increased egg production as being the result of optimal diet mixing or not (Cock 1978; Sabelis 1990). For this, tedious measurements of encounter rates with both prey and success rates of attacks would be needed, both on single diets and on mixed diets. Here, we have used an alternative experimental solution, focusing directly on changes in the predator's patch-commuting frequency when the two prey species were offered on two separate, connected patches. This allowed us to use an additive design without the associated changes in prey density that would occur otherwise. Hence, the increases in oviposition rate observed here were not due to a higher density of food (each patch had the same prey density), but to the availability of two different food types. If the two prey species would be of similar quality, foraging on the two patches would result at best in an equal or slightly lower oviposition rate because the predators would spend time and energy on commuting. Similar experimental designs can prove useful for assessing functional responses on mixtures of prey types. Thus, we have shown conclusively that two predatory mite species

actively forage to obtain such a mixed diet and benefit from it.

Although we did not observe differences in the frequency with which the mites crossed the bridge between the two patches during the first 5 h of the experiment, predation occurred more frequently on both patches when each contained a different prey species than when both patches harboured the same prey. We do not know how the predators perceived that the two patches contained different prey; perhaps they needed to sample both patches first, which would explain the lack of difference in the frequency with which they moved from one patch to the other during the first 5 h of the experiment.

When the two prey species were offered on separate patches, we used the eggs of the two prey species for four reasons. One is that mobile prey stages can migrate to the patch with the other prey species. Moreover, mobile stages of the two prey species may also interact after having moved to the other patch. For example, one of the two prey species that constitutes a mixed diet for the predatory mite *Amblyseius swirskii* (Messelink *et al.* 2008) also feeds on the other prey species (Van Maanen *et al.* 2012). Thirdly, mobile prey often display antipredator behaviour, and this is affected by the diet of the predator (Mathis & Smith 1993a,b; Venzon *et al.* 2000; Persons *et al.* 2001; Van Maanen *et al.* 2015). As a consequence, the optimal diet of a predator may be confounded by the amount of feeding on each of the prey types, and the response of the prey to diet cues associated with the predator (Venzon *et al.* 2000; Persons & Rypstra 2001) or alarm cues of conspecific prey (De Bruijn *et al.* 2006). Fourthly, using immobile prey (eggs) on separate patches largely prevents that they can influence each other's vulnerability to the predator or each other's digestibility to the predator (Lesna & Sabelis 1999). Whereas all these factors are possibly important in determining the diet of predators in the field, diet mixing and self-selection are, at least initially, best studied without them.

Animals will not always find foods of different composition in the same patch, so they will need to commute between patches with different food items to mix their diet. For herbivores, several studies on diet mixing have considered such spatial separation of the various diet elements. For example, Mody, Unsicker & Linsenmair (2007) show that specialist caterpillars perform better on a mixture of foliage of several individuals of the same tree species than on foliage of single trees and that they cover several metres to disperse to other host plant individuals. Behmer *et al.* (2003) showed that the distance between two different foods did not have a significant effect on the intake of proteins and carbohydrates in a locust. Hence, both these studies show no effect of distance between diet items on the diet mixing of herbivores. However, it is likely that there will be a maximum to the distance that animals are willing to commute to obtain a mixed diet. Bridging this distance will probably be associated with costs that offset the advantages of a mixed diet. Our experiment with the

two prey species on different patches can be viewed as a first step to identify a trade-off between feeding on a mixed diet and the costs associated with obtaining it in predators. Our results suggest that the distance between the two patches was not sufficient to induce significant costs that would result in decreased oviposition or significant changes in the diet composition. Future experiments should study the propensity of animals to cover larger distances to feed on a mixed diet.

Albeit only for two species of plant-inhabiting predatory mites, our results provide support for active diet mixing of prey as an explanation for polyphagy in predatory arthropods. We suspect that more polyphagous predator species will show such an ability to balance their nutrients or dilute toxins by feeding on more than one prey species. This would then represent an alternative to the hypothesis that polyphagy arises from local populations consisting of mixtures of predators that are each specialized on a limited range of prey (Fox & Morrow 1981; Tauber *et al.* 1993; Lesna & Sabelis 1999, 2002).

Acknowledgements

The authors thank two anonymous reviewers for valuable comments. AJ thanks Thomas van Wissen for computer support. The National Council of Technological and Scientific Development, Brazil (CNPq), provided financial support (projects 620028/2008-4; 475408/2008-0 and 303523/2012-3) and the Federal Agency for Support and Evaluation of Graduate Education (CAPES) provided a master scholarship to the first author. The National Postdoctoral Programme (CAPES-PNPD) provided a postdoc scholarship to MP-N. AJ received a scholarship from FAPEMIG (CBB-3000309), MV and RS received a scholarship from CNPq. The authors declare no conflicts of interest.

Data accessibility

Data are deposited in the Dryad Digital Repository: doi: 10.5061/dryad.bv247 (Marques *et al.* 2015).

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Received 16 April 2014; accepted 26 February 2015

Handling Editor: David Raubenheimer