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Assessing the impact of long-term rearing on pollen diet (saffron and cattail) versus factitious prey [*Tyrophagus putrescentiae* (Acari: Astigmatidae)] on the biological performance of *Neoseiulus californicus* (Acari: Phytoseiidae)

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ABSTRACT

The biological performance of *Neoseiulus californicus* (McGregor) as a selective generalist predator of spider mites was assessed up to 10 generations (G1 and G10) fed on the saffron and cattail pollen grains, as well as the factitious prey *Tyrophagus putrescentiae* Schrank (Acari: Astigmatidae). All the experiments were conducted under laboratory conditions at 25±1°C, 65±5% RH, and a photoperiod of 16:8 (L: D) h. The total pre-oviposition period (TPOP) was shorter in G10 than in G1 when the predator was fed with the factitious prey. Female longevity and the total lifespan of *N. californicus* were significantly reduced in G10 compared to G1 across all diets. The values of the gross reproductive rate (*GRR*), net reproductive rate (R_0), and intrinsic and finite rates of increase (r and λ, respectively) had no significant difference between G1 and G10 when the predator was reared on the cattail pollen. In contrast, the values of these parameters were significantly lower in G10 on the prey and saffron pollen. After one generation of feeding on saffron pollen, *GRR*, R_0, r, and λ showed no significant differences compared to the cattail pollen, prey, and mixed diets, while in G10, these values had no significant difference when the predator reared on the cattail pollen and factitious prey, whereas these values were higher than those fed on the saffron pollen. In conclusion, cattail pollen and *T. putrescentiae* are more suitable than saffron pollen for the rearing of the predatory mite *N. californicus* for up to 10 generations.

Keywords: factitious prey, mass rearing, mold mite, predatory mite, two-sex life table.

INTRODUCTION

Biological control provides an environmentally safe, cost-effective, and energy-efficient means of pest control, which has been widely used since the end of the 19th century. In augmentative biological control, natural enemies are mass-reared in biofactories for augmentative release to obtain immediate control of pests (van Lenteren, 2012). *Neoseiulus californicus* (McGregor), a Mediterranean-native phytoseiid mite, was first described in 1954 on lemon in California (de

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Moraes *et al*., 2004). This species has characteristics of both type II specialist predators (McMurtry *et al*., 2013), and shows a Type II functional response in feeding on *T. urticae* (Farazmand et al., 2012). *Neoseiulus californicus* is one of the major predators of spider mites and *Tetranychus* species; however, it can consume other mite species and some insects (Yazdanpanah and Fathipour, 2023a). In addition, *N. californicus* can survive on a diet of pollen. The use of pollen has been shown to facilitate rearing and reduce the costs of mass production of phytoseiid mites. In addition, pollen grains are used as a dietary supplement or alternative food by predatory mites when primary prey is scarce (Al-Shammery, 2011). The pollen grains of castor bean (Marafeli *et al*., 2014), pistachio, apricot (Soltaniyan *et al*., 2018), thorn apple (Eini *et al*., 2023), and oak (Simoni *et al*., 2023) are suitable alternative diets for the mass rearing of *N. californicus*. While pollen offers essential nutrients, using more affordable and readily available food sources like factitious prey could enhance the efficiency of phytoseiid mite mass production (Yazdanpanah *et al*., 2022).

Mites of the cohort Astigmatina are suitable factitious prey for several phytoseiid species, and most of them can be easily produced in large numbers on flour, bran, or similar substrates while maintained in relatively small containers (Barbosa and Moraes, 2015). The astigmatid mites usually render the rearing process less expensive than those using phytophagous mites as food due to reduced requirements for space, labor, and maintenance costs (Gerson *et al*., 2003). The importance of some astigmatids such as *Carpoglyphus lactis* L., *Lepidoglyphus destructor* (Schrank), *Tyrophagus putrescentiae* Schrank (Tung *et al*., 2022), *Glycyphagus domesticus* (De Geer) (Simoni *et al*., 2023), *Austroglycyphagus lukoschusi* (Fain), and *Blomia tropicalis* Bronswijk, de Cock and Oshima (Barbosa and Moraes, 2015) has been studied for mass production purposes.

Insect demography, the study of insect population dynamics, is crucial for understanding ecological systems, pest management, and the conservation of beneficial insects. Demography brings life table techniques, mortality models, experimental systems, and comparative methods that enhance our ability to analyze and predict insect population dynamics. This is important in entomology because it provides a comprehensive and reliable statistical basis for life table and mortality analysis. (Carey, 2001). Although *N. californicus* performance has been evaluated with pollen and factitious prey diets (Bellutti, 2011; Sorensen *et al*., 2012), the long-term effects of such diets on the predator's biology remain insufficiently explored. Therefore, in the present study, the

biological performance of *N. californicus* was assessed after long-term feeding on saffron and cattail pollen grains, as well as the factitious prey *T. putrescentiae*.

MATERIALS AND METHODS

Pollen collection

Pollen of saffron (*Crocus sativus* L., Iridaceae) was collected in autumn from Khorasan Razavi province, Northeastern Iran. Cattail (*Typha latifolia* L., Typhaceae) pollen was collected in early summer from Dorud, Lorestan province, Western Iran. These pollens were selected because of their storage capacity without decreasing nutritional quality (Moradi *et al*., 2021) and less labor cost for their collection in our country. The pollen grains were dried at 28°C for 24 h and stored in a refrigerator set at 4°C for a short period (one week) until used in the experiments and frozen at -18°C for long-term (two months) usage.

Stock culture of the stored product mite, *Tyrophagus putrescentiae*

The individuals of *T. putrescentiae* were originally collected from the infested stored products such as flour and seeds in Tehran, Iran. After identifying the species by using relevant keys (Noei *et al*., 2012), they were reared on wheat bran and flour at 27±1°C, 70% ±5 RH, and a photoperiod of 8:16 h (L:D) in a fine-mesh covered Plexiglas container (10×7×4 cm) for about 20 days.

Stock culture of *Neoseiulus californicus*

To establish the stock culture, *N. californicus* individuals were received from Biobest, Belgium. To establish a laboratory colony of *N. californicus*, the individuals (about 300 of all stages) were transferred onto a green plastic sheet (16×11×0.1 cm) sitting atop a water-soaked sponge in a Plexiglas container (30×15×12 cm). All plastic sheet edges were covered using moist tissue paper, which acted as a barrier, prohibiting mites from escaping. The stock culture was kept in a growth chamber at 25±1°C, 60±5% RH, and a photoperiod of 16L:8D h. The new pollen of saffron and cattail (separately) at 4-day intervals and a mixture of immature stages of *T. putrescentiae* (2000-3000 individuals) once a week was offered as food sources over 10 generations, and a combination of cattail pollen + *T. putrescentiae* was offered once a week for one generation.

Experimental setup

The experimental units were similar to the stock culture rearing units but smaller, consisting of green plastic sheets (3× 3× 0.1 cm), plastic trays (7×5×4 cm), and wet sponges. Some cotton fibers were stuck onto the plastic sheet to provide a suitable place for oviposition. To supply the moisture needed for the mites, the edges of the sheet were covered with moist tissue paper, and water was added daily to prevent the strips from drying out.

To ensure providing the same-aged eggs of *N. californicus*, more than 40 pairs of the predator were selected randomly from the stock culture for each generation and kept in a new experimental unit for less than 24 h. The eggs laid by the females were then transferred to the experimental units. After larval emergence, they were fed individually with 1 microgram of pollen, and 80 (all stages) factitious prey, or a mixed diet. Immature development time and survival were recorded daily until they reached adulthood. After adult emergence, the females were coupled with the males of the same treatment. Daily monitoring was continued until the adults' death. Oviposition, survival, adult longevity, and oviposition periods of the mites were recorded daily. For all treatments, G1 had no experience of tested diets, and individuals were obtained from the stock culture. The predator individuals were reared on the respective diets of saffron pollen, cattail pollen, and *T. putrescentiae* for 10 generations and prey + cattail pollen for one generation. All the experiments were conducted at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 8:16 h (L:D) at the first and 10th generations. In all replicates (about 40), ad-lib new pollen (≈1 microgram) was added at 4-day intervals, and adequate different stages of the prey (50-60) were offered once a week.

Data analysis

Life table parameters included *GRR*, gross reproductive rate [total lifetime reproduction (number of offspring per individual) in the absence of mortality]; R_0, net reproductive rate [total lifetime reproduction (number of offspring per individual), taking normal mortality into account.); *r*, intrinsic rate of increase [represents the difference between the intrinsic birth rate and death rate inherent in population per individual per day]; λ, finite rate of increase [gives the proportional change in population size from one time period to the next]; and *T*, mean generation time [the time length that a population needs to increase to R_0-times of its size as the stable age distribution and the stable increase rate are reached]. Life table parameters as well as, age-stage-specific population structure (p_{xj}) (the proportion of individuals at age *x* and stage *j* in the environment; age-stage

structure), age-specific survivorship (l_x) (the probability that a newborn will survive to age x, calculated by pooling all of the surviving individuals of different stages), age-stage-specific fecundity (f_{xj}) (eggs produced per female per day), and age-specific fecundity (m_x) (eggs produced per individual per day) were calculated according to the age-stage, two-sex life table procedure (Chi and Liu, 1985; Chi, 1988) using the TWOSEX MSChart software (Chi, 2025) at different generations on different diets. The variances and standard errors of the life table parameters were determined by the bootstrap procedure (100,000 samples) (Huang and Chi, 2012). Multiple comparisons between different generations of each diet and the corresponding generation among different diets were carried out using the paired bootstrap test (Reddy and Chi, 2015). The most important equations are as follows:

$GRR = \sum_{x=\alpha}^{\beta} m_x$

Gross reproductive rate (eggs per individual)

$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$

The net reproductive rate (eggs per individual)

$r \sum_{x=0}^{\omega} e^{-r(x+1)} l_x m_x = 1$

Intrinsic rate of increase (day^{-1})

$\lambda = e^{r}$

Finite rate of increase (day^{-1})

$T = \frac{\ln R_0}{r}$

Mean generation time (day)

RESULTS

Life table parameters of *N. californicus* in different generations on each diet

No significant difference in the total pre-adult duration was observed between G1 and G10 of the predator by feeding on both pollen types, whereas the developmental time was recorded significantly shorter in G10 than G1 when the predator was fed on the factitious prey (*T. putrescentiae*). Male longevity was not significantly different across generations, except under the saffron pollen diet, where males in G10 lived shorter than those in G1. Female longevity and the total life span (from birth to death) of *N. californicus* after 10 generations were significantly shorter than the first generation on all diets. Adult pre-oviposition period (APOP) did not differ among

generations of the three tested diets, while the total pre-oviposition period (TPOP) was shorter in G10 than G1 by feeding on the factitious prey. In G10, oviposition days and fecundity were reduced compared to G1 for all diets tested. The immature survival did not differ between generations on the cattail and prey diets, but in G10, it was less than G1 when the saffron pollen was offered (Table 1). The values of the gross reproductive rate (*GRR*), net reproductive rate (R_0), intrinsic and finite rates of increase (r and λ, respectively) had no significant difference between G1 and G10 when the predator was reared on the cattail pollen, while the values of these parameters were significantly lower in G10 on the prey and saffron pollen as diets (Table 2).

Life table parameters of *N. californicus* in the corresponding generations of diets

In the corresponding generations, the developmental time had no significant difference between all tested diets in G1, however, this parameter in G10 was shorter by feeding on the factitious prey (*T. putrescentiae*). Rearing the predator on the saffron pollen led to the longest total lifespan, and after that, the cattail pollen, prey, and finally the combination diet (prey + cattail pollen) were in the next levels in G1. After 10 generations, the total lifespan had no significant difference between the saffron pollen and prey, while this was shorter than those reared on the cattail pollen. APOP and TPOP had no significant difference between all treatments in G1, while these parameters were shorter in G10 by feeding on the factitious prey. The lowest fecundity and oviposition days in G1 belonged to the predators reared on the cattail pollen and a mixed diet, and in G10, the mentioned parameters had no significant difference between the population fed by prey and those reared on pollen diets (Table 1). When the predator fed on the saffron pollen for one generation, the values of *GRR*, R_0, r, and λ had no significant difference compared with the cattail pollen, prey and prey + pollen diets, however, the results showed higher values of these parameters by feeding on the factitious prey than the cattail pollen in G1. In G10, the values of *GRR*, R_0, r, and λ had no significant difference when the predator reared by the cattail pollen and prey, whereas these values were higher than those on the saffron pollen (Table 2).

Age-stage-specific population structure (p_{xj}) of *N. californicus* fed in the first and 10th generations on different diets is shown in Figure 1. The adult stage began around the age of 5 days in both G1 and G10 when the diet was the cattail pollen, and 6 days in G1 and G10 for the other diets. In addition, both females and males in G1 lived more than those in G10 (Figure 1). Based on the fecundity curves, the peak of fecundity in G1 was 2.53 eggs/female at the age of 14 days when the

factitious prey was offered, and in G10, it was 2.36 eggs/female at the age of 15 days by feeding on the cattail pollen (Figure 2).

DISCUSSION

Our results showed that *N. californicus* could feed and develop into adults on all diets tested. In arthropods, the duration of egg and larva are affected by the mother's nutrition (Vijendravarma *et al*., 2010), but these periods of the predatory mite were not affected by the long-term feeding of females, because there was not any significant difference among different diets and generations for the periods of egg and larva. In addition, other immature stages were not affected by long-term rearing on both pollen grains; therefore, there was a non-significant difference between the durations of total developmental times. The prey *T. putrescentiae* was a higher nutrient diet for protonymphal and deutonymphal stages compared with pollen, especially in long-term rearing because its pre-adult period in G10 was shorter than that in G1. A shorter pre-adult period is a good feature for predators because the predation capacity of immatures is less than that adult, while with the quick emergence of the adults, predation rate, and finally, the consequent control on the target pest may increase (Yazdanpanah *et al*., 2021).

Short developmental time leads to a shortened generation duration and consequently increases the population growth potential of a predator. In the current study, the TPOP as an index for a generation duration, by feeding on pollen and factitious prey in G1 (9-10 days) was shorter than that when *N. californicus* fed on *Austroglycyphagus lukoschusi* and *Blomia tropicalis* (15 days) (Barbosa and Moraes, 2015), *T. putrescentiae* (18 days) (Khanamani *et al*., 2021), and the main prey *Tetranychus urticae* Koch (11 days) (Khanamani *et al*., 2017). The total lifespan of the predatory mite by feeding one generation on the saffron pollen (67 days) and *T. putrescentiae* (34 days) was longer than that reported by Eini *et al*. (2022) (50 days on the saffron pollen) and Khanamani *et al*. (2021) (27 days on *T. putrescentiae*). The fecundity of *N. californicus* on *T. putrescentiae* (5.71 eggs/female) (Khanamani *et al*., 2021) was lower than the currently recorded fecundity, but when the predator fed on the saffron pollen (46.12 eggs/female) (Eini *et al*., 2022), *L. destructor* (51.48 eggs/female), *T. urticae* (45.11 eggs/female), and *C. lactis* (38.68 eggs/female) (Tung *et al*., 2022), the recorded fecundity was higher than our results in the first generation. The comparison of the biological parameters of *N. californicus* among different research works is complicated because of differences in experimental conditions, as well as the

type of data analysis, different population genetics of a predator and prey, and different methods of pollen collection. In addition, the use of different food substrates for factitious prey may produce different results when they are offered to the predator; therefore, the rearing methods of the prey may also influence a predator's performance (Huang *et al*., 2013).

Decreased female longevity and oviposition days in G10 contributed to reduced fecundity compared to G1, which means these parameters are affected by long-term rearing on alternative diets. The other important parameters, such as the net reproductive rate (R_0) and the intrinsic rate of increase (r), are also affected by long-term rearing on the diets. In G10, the values of R_0 and r parameters were lower than G1 by feeding on *T. putrescentiae* and saffron pollen, which indicated that the predatory mite was affected by long-term rearing on both saffron pollen and *T. putrescentiae*, whereas the predator's performance was stable by long-term feeding on the cattail pollen.

Diets for the mass rearing of biocontrol agents could also be expanded to include a much wider range of food sources by combining specific nutrients (Wade *et al*., 2008). Diet mixing is common to rear the generalist predatory mites, and the benefits deriving from being generalist have been mainly attributed to the ability of several species to feed on different types of prey containing different amounts of nutrients and thus actively restore nutritional imbalances in their diets (Mayntz *et al*., 2005). Positive effects of diet mixing on phytoseiids' performance have already been documented (Samaras *et al*., 2019; Mortazavi *et al*., 2023; Yazdanpanah and Fathipour, 2023b). A mixed diet, including a combination of two or several prey, a combination of prey and pollen, and a mixture of different pollen grains could enhance the biocontrol effectiveness because of the positive effects of this diet type on the numerical response of predators (Delisle *et al*., 2015). In the present study, the pollen grain of cattail was used in a mixed diet because of its stable intrinsic rate of increase and more affordable diet. Soltaniyan *et al*. (2020) indicated high population growth parameters of *N. californicus* when fed on a mixed diet of pistachio pollen + *T. urticae*. In addition, Xin and Zhang (2021) reported that the mixture of the cattail pollen and the eggs of *Trialeurodes vaporariorum* (Westwood) were suitable for different developmental stages of *Amblyseius herbicolus* (Chant). In contrast, the performance of the predator in the current study almost was not affected by rearing on a mixed diet of cattail pollen and *T. putrescentiae* compared with feeding on pollen or factitious prey separately.

In conclusion, both pollen grains of cattail and saffron, as well as the factitious prey *T. putrescentiae* are suitable diets for the rearing of the predatory mite *N. californicus* up to 10 generations. However, the performance of the predatory mite was not affected by the mixed diet of pollen + prey compared with feeding on these diets alone. To make a definitive decision about the best diet, it is necessary to monitor the predatory performance for a longer period and then conduct greenhouse experiments in the presence of the main prey.

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364

Table 1. Long-term effects of feeding on pollens of cattail and saffron, and the factitious prey of *Tyrophagus putrescentiae* on duration of different life stages (days), oviposition days, and fecundity (eggs/female) (mean ± SE) of *Neoseiulus californicus.*

Parameter	Cattail (G1)	Cattail (G10)	Saffron (G1)	Saffron (G10)	Prey (G1)	Prey (G10)	Cattail +Prey
Egg (days)	1.57±0.159aB	1.79±0.176aA	2.30± 0.103aA	2.00±0.198aA	2.26± 0.101aA	1.77± 0.177bA	2.00±0.144A
Larva (days)	1.00±0.000aA	1.00±0.000aA	1.00±0.000aA	1.00±0.000aA	1.00±0.000aA	1.00±0.000aA	1.00±0.000A
Protonymph (days)	2.45± 0.111aA	2.42± 0.113aA	1.90±0.067aB	2.11± 0.104aB	2.05±0.051aB	2.07±0.123aB	2.00±0.000B
Deutonymph (days)	2.26± 0.126aA	2.00± 0.130aB	2.05±0.086AB	2.62±0.173aA	1.83±0.088abB	1.77±0.162aB	1.92±0.075B
Pre-adult (days)	7.26±0.234aA	7.21±0.177aA	7.25±0.097aA	7.62±0.352aA	7.17±0.088aA	6.54±0.177bB	7.00±0.110A
Male longevity (days)	30.71±3.016aAB	22.33±3.552aA	50.83±14.207aA	7.78±2.182bB	20.20±4.457aB	11.25±3.061aB	19.30±7.378B
Female longevity (days)	35.76±4.096aB	23.43±4.223bA	68.55±8.578aA	14.00±0.915bB	28.46±2.354aBC	14.30±1.560bB	21.65±3.468C
Total lifespan (days)	41.17±2.758aB	30.37±3.088bA	67.83±7.959aA	17.82±1.825bB	34.39±2.055aC	20.23±1.281bB	27.99±3.080C
APOP (days)	2.73±0.297aA	3.08±0.254aA	2.36±0.149aA	6.34±2.605aA	2.53±0.130aA	2.22±0.143aB	3.33±0.961A
TPOP (days)	10.36±0.531aA	10.25±0.363aA	9.55±0.242aA	13.67±2.909aA	9.73±0.177aA	8.89±0.254bB	10.22±0.957A
Oviposition days (days)	15.19±1.129aBC	11.00±1.281bA	23.82±1.586aA	5.33±1.224bB	17.26±1.409aB	8.33±1.356bAB	11.22±1.790C
Fecundity (eggs/female)	25.26±2.859aB	17.00±2.733bA	40.10±3.023aA	8.00±1.835bB	33.06±2.782aAB	11.89± 2.193bAB	21.99±3.983C
Immature survival (%)	90±0.064aAB	90±0.064aA	100±0.000aA	66±0.133bA	90±0.067aAB	76±0.102aA	76±0.103B

G, generation; Prey, *Tyrophagus putrescentiae*; APOP, adult pre-ovipositional period (from adult emergence to first oviposition); TPOP, total pre-ovipositional period (from egg to the first oviposition).

The means followed by different lowercase letters in the same row among different generations (G1 and G10) of each diet are significantly different. The means followed by different capital letters within the same row between the corresponding generations of different diets are significantly different ($P < 0.05$, paired-bootstrap test).

Table 2. Long-term effects of feeding on pollens of cattail and saffron, and the factitious prey of *Tyrophagus putrescentiae* on the life table parameters (mean ± SE) of *Neoseiulus californicus.*

Parameter	Cattail (G1)	Cattail (G10)	Saffron (G1)	Saffron (G10)	Prey (G1)	Prey (G10)	Cattail +Prey
***GRR* (eggs/individual)**	16.53±3.492aB	16.24±3.060aA	25.40±4.997aAB	5.85±2.728bB	32.21±3.818aA	12.27±2.390bAB	19.96±5.395AB
R_0 (eggs/individual)	14.44±3.168aB	11.34±2.509aA	22.06±4.739aAB	2.07±1.035bB	24.79±3.829aA	6.99±1.905bA	11.65±3.350B
***r* (day^{-1})**	0.153±0.015aB	0.148±0.016aA	0.162±0.014aAB	0.037±0.036bB	0.191±1.042aA	0.139±0.021bA	0.158±0.021AB
λ (day^{-1})	1.166±0.018aB	1.160±0.018aA	1.177±0.017aAB	1.039±0.037bB	1.210±0.013aA	1.149±0.024bA	1.171±0.024AB
***T* (day)**	17.27± 0.713aAB	16.21±0.601aA	18.92±0.526aA	17.34±3.007aAB	16.74±0.362aB	13.74±0.564bB	15.24±0.604C

G, generation; Prey, *Tyrophagus putrescentiae*; *GRR,* gross reproductive rate; R_0, net reproductive rate; *r,* intrinsic rate of increase; λ, finite rate of increase; *T,* mean generation time.

The means followed by different lowercase letters in the same row among different generations (G1 and G10) of each diet are significantly different. The means followed by different capital letters within the same row between the corresponding generations of different diets are significantly different ($P < 0.05$, paired-bootstrap test).

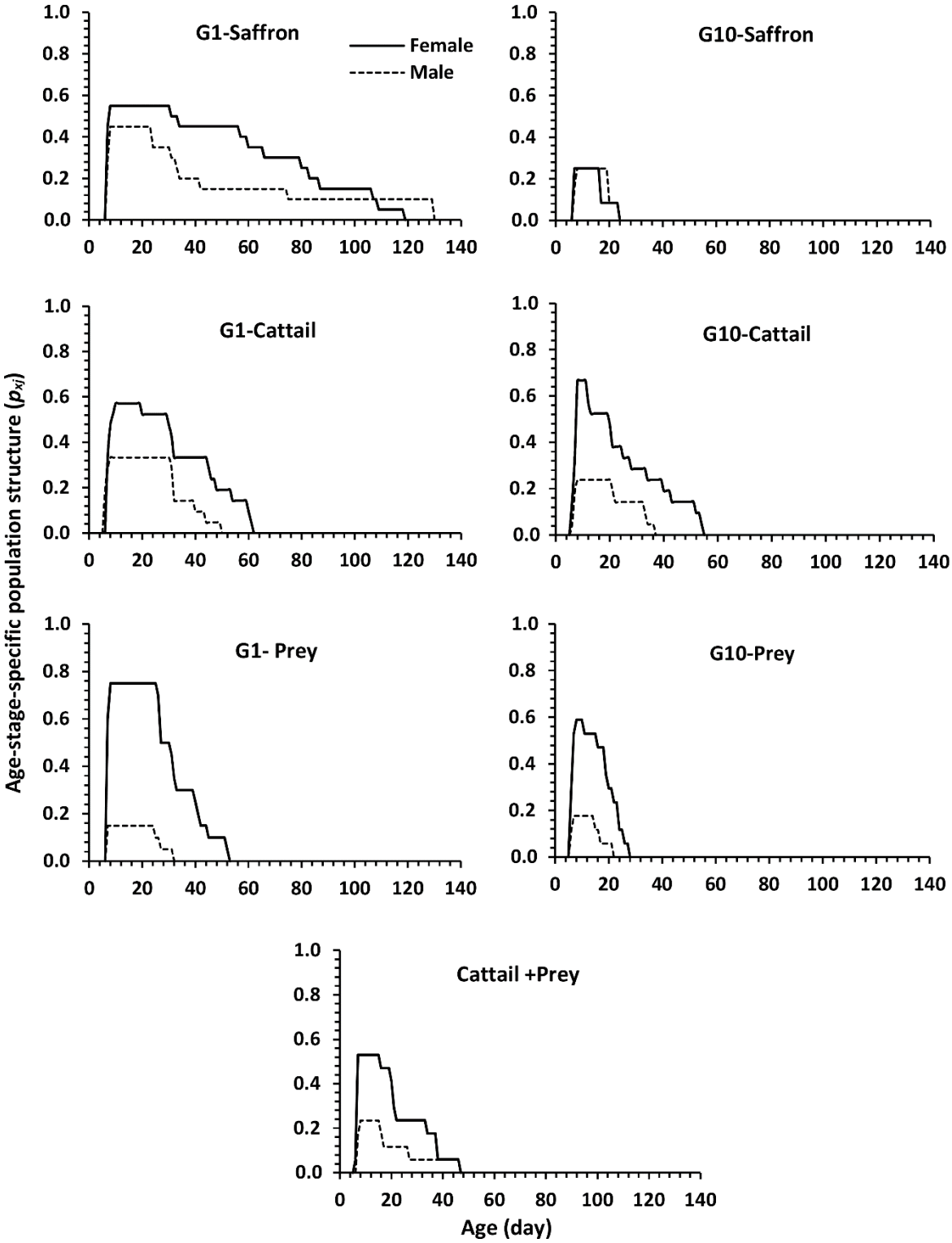

Figure 1. Age-stage-specific population structure of sequential generations (G1-G10) of *Neoseiulus californicus* reared on saffron pollen, cattail pollen, prey (*Tyrophagus putrescentiae*) and mixed diet (cattail pollen+ prey).

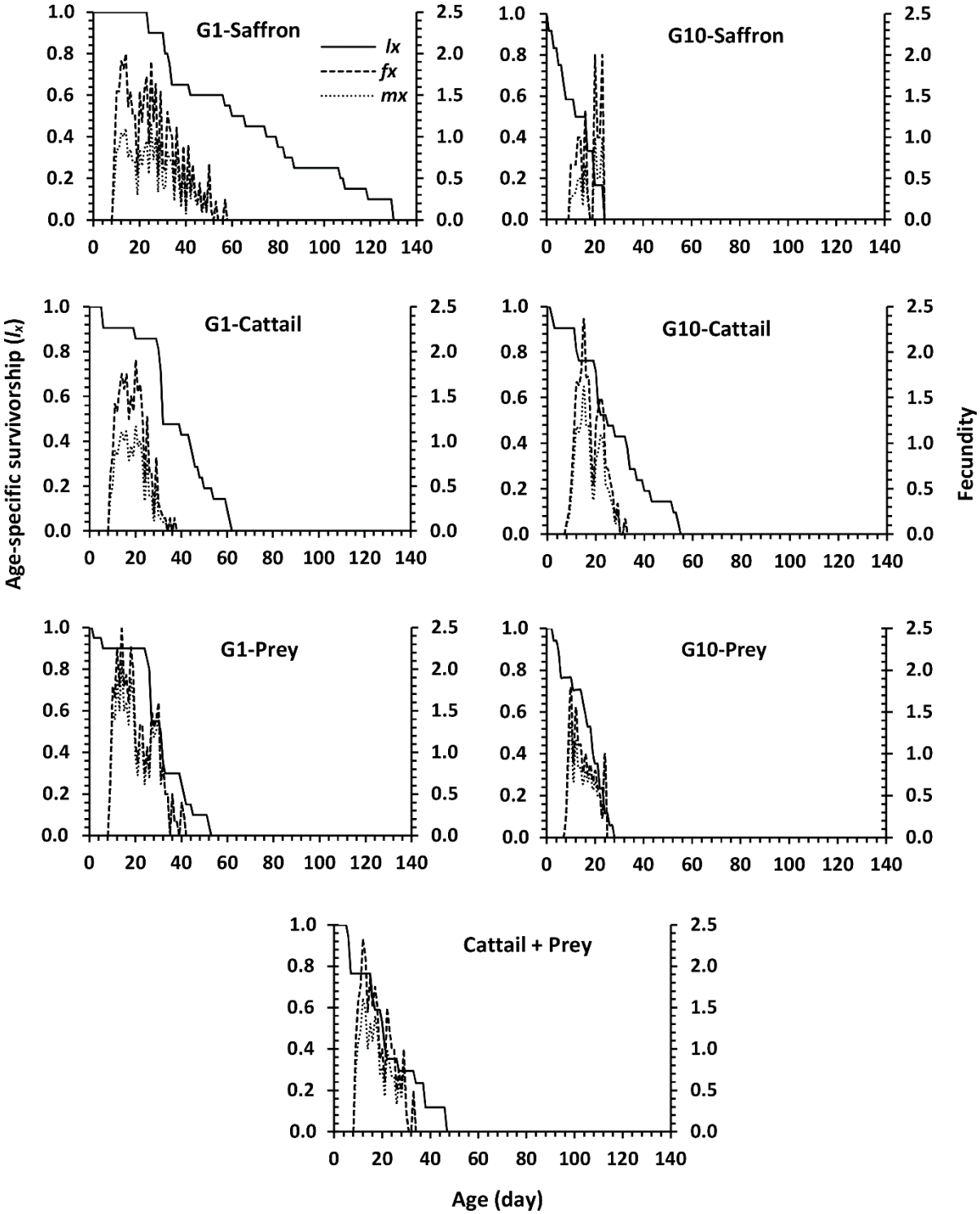

Figure 2. Age-specific survivorship (l_x), age-specific fecundity (m_x), and age-stage-specific fecundity (f_{xj}) of sequential generations (G) of *Neoseiulus californicus* reared on saffron pollen, cattail pollen, prey (*Tyrophagus putrescentiae*), and the mixed diet (cattail pollen + prey)