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Social environment refers to an individual's physical surroundings, community resources and social relationships, including population size, population composition, fission-fusion dynamics, and the social interactions among group members (Webber *et al.* 2022). Population size and density are two key socio-environmental factors that affect the offspring sex ratio in group-living animals. For example, in small populations founded by one or a few foundresses, females tend to produce female-biased offspring to reduce the local mate competition between related males (Hamilton 1967; Krainacker & Carey 1991; Roeder 1992; Sato & Saito 2006; West 2009; Macke *et al.* 2012, 2014), whereas in dense populations mothers incline to produce more offspring of dispersive sex to reduce resource competition (Clark 1978; Mari *et al.* 2008; Hjernquist *et al.* 2009; Iritani *et al.* 2021; Weerawansha *et al.* 2022a, b). Social environments may affect offspring sex ratio directly by altering mothers' sex allocation strategies (Clark 1978; Iritani *et al.* 2021; Weerawansha *et al.* 2022a, b) or indirectly through mediating their other life history traits such as clutch size (West 2009) and egg size (West 2009; Macke *et al.* 2011, 2012).

Moreover, previous studies reveal that in haplodiploid species (i.e., whose fertilized eggs give rise to diploid female offspring and unfertilized eggs to haploid male offspring), life history traits such as the maternal age can also directly affect sex allocation due to the age-specific fertilization (i.e., more daughters produced at specific ages) (Roy *et al.* 2003; Ueno 2014; Weerawansha *et al.* 2020). Nevertheless, maternal age may also affect offspring sex ratio indirectly through age-specific investment in clutch size (Weerawansha *et al.* 2020) and egg size (Reviewed in Mousseau & Fox 1998). Therefore, multiple factors of social environments and life history traits may simultaneously act on offspring sex ratio during the females' reproductive period. However, studies usually test the influence of social environments and life history traits on sex allocation separately and focus on the direct effect only (West 2009; Macke *et al.* 2011, 2012; Katlav *et al.* 2021). Due to the lack of appropriate methodology, few studies have investigated (1) whether social environments and maternal age have both direct and indirect effects on sex allocation, and (2) whether social environments impose weaker or stronger effects on offspring sex ratio than the life history traits. Here we used a haplodiploid spider mite *Tetranychus ludeni* Zacher (Acari: Tetranychidae) as a model species to estimate and compare the effects of social environments and life history traits on sex allocation.

We established a colony of *T. ludeni* Zacher from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand. The colony was maintained, and the experiment was carried out at 25 ± 1°C and 40–50% RH with a photoperiod of 16L:8D hrs. We used the first

expanded leaves of 1- to 2-week-old bean plants (*Phaseolus vulgaris* L.) for the experiment. To estimate the effects of social environments and life history traits on sex allocation in *T. ludeni*, we designed a factorial experiment by allowing both population size and density to vary, with nine combined treatments: three population sizes (1, 5, and 10 ovipositing females) at three population densities (0.67, 1, and 2 ovipositing females/cm^2). To obtain mated females, we randomly collected the quiescent female deutonymphs from the colony and individually placed them on a 1-cm^2 leaf square placed upside down on a wet cotton pad in a Petri dish (9.5 cm diameter×1.0 cm height, with a 1-cm mesh-sealed hole in the middle of the lid). Before the female emerged, we introduced a newly emerged male adult into the mating arena. The males were produced by virgin females that developed from individually reared quiescent female deutonymphs randomly collected from the colony. Mating occurred at female emergence and usually lasted for < 2 mins (NW personal observations).

For each replicate, we transferred mated female(s) of a desired population size or density onto a leaf square of the desired size (Table 1) in a Petri dish mentioned above and allowed them to lay eggs for 24 hours. After which time, we then transferred them to a new leaf square of the same size daily until they died. We counted the number of eggs laid on each leaf square, and measured egg diameter under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan), using an imaging software (CellSens® GS-ST-V1.7, Olympus, Japan). We calculated the egg size: volume=$4/3\pi r^3$, where *r* is radius (=diameter/2). As up to 10, 35, and 85 eggs could be laid on a leaf square by 1, 5, and 10 females, respectively, we measured all, 10 and 15 eggs on leaf squares with 1, 5 and 10 females feeding on, respectively. We allowed the larvae and nymphs to feed on the same leaf square for five days, after which time they were transferred onto another clean fresh leaf square of the same size where they developed to adults. The number and sex of emerged adults were also recorded and the offspring sex ratio [daughter%=daughters / (daughters + sons)] was calculated for each leaf square. We only used data recorded during the first five days of the oviposition period for analysis, because the social environment changed beyond day 5 of oviposition due to the death of experimental female mites.

We analysed the data using SAS 9.4 (SAS Institute Inc., Cary, NC). A path analysis (CALIS Procedure) was applied to estimate the direct effects of population size and density, their interaction, maternal age, and egg number and size on sex ratio, whereas the indirect effects of population size and density, their interaction, or maternal age on sex ratio were assessed through their influences on egg number and size (Figure 1). We then used a multivariate linear regression model (REG procedure) to compare the direct effects of social environments and life history traits, according to the partial regression coefficient (R^2, measuring the proportion of variance in sex ratio that can be explained by a given factor, the larger the R^2, the more variance could be explained) of each factor (Table 2). The partial R^2 of each factor was calculated as the Type I Sum of Square of a factor divided by the Uncorrected Total Sum of Square of the model. Using the same multivariate linear regression model, we employed the stepwise-selection method to organise the significant factors in order by adding them one by one into the model, with the most significant factor entering the model first and the non-significant ones being excluded from the final model (Table 2). To detect the cause of the positive effect of egg number on sex ratio, a linear regression (REG procedure) was used to determine the relationships between the number of eggs and daughters/sons produced, and the significant difference in regression coefficient (i.e., slope) of regression lines was indicated by the non-overlapped 95% CLs of coefficients. As the measuring scales and units of different factors were highly different, we standardised each factor as well as data on offspring sex ratio to have a mean of 0 and a standard deviation of 1 (STANDARD procedure) before path analysis and multivariate regression, so that all factors were comparable.

TABLE 1. Experimental design for nine combined treatments of three population sizes at three population densities.

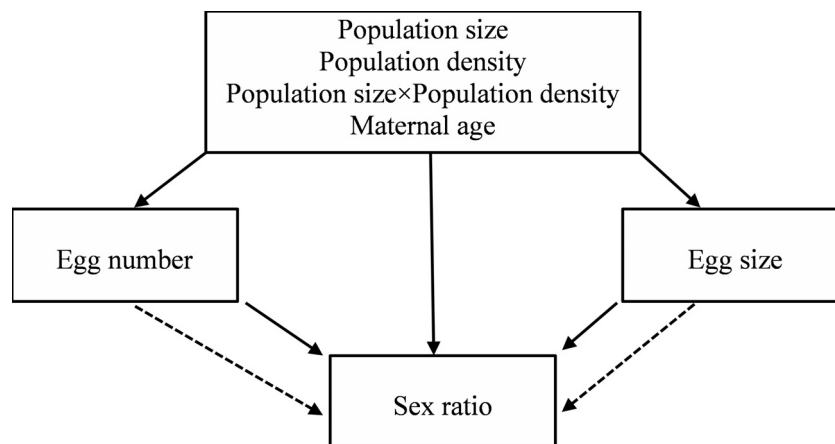| Leaf area (cm^2)	Population size [female(s)]	Population density [female(s)/cm^2]	Replicate (n)
1.5	1	0.67	25
1	1	1	18
0.5	1	2	24
7.5	5	0.67	25
5	5	1	22
2.5	5	2	16
15	10	0.67	18
10	10	1	16
5	10	2	21

FIGURE 1. A diagram showing the paths of direct (solid line) and indirect (dashed line) effects of social environments [population size and density and their interaction (Population size×Population density)] and life history traits (maternal age, egg number, and egg size) on sex allocation in *Tetranychus ludeni*. Arrows of solid and dash lines indicate the directions of direct and indirect effects, respectively.

Due to the possible correlations of population size and density and maternal age with the egg number and size, multicollinearity might exist, which could result in inaccurate interpretations of the effects of explanatory factors as the change of one factor would inevitably lead to the change of another (Tay 2017; Frost 2019). We thus examined the variable multicollinearity through the variance inflation factor (VIF) in the above-mentioned multivariate linear regression model. The VIF measures the inflation in the variances of factors. Results show that the VIF=1.09 ~ 1.03, indicating a lack of multicollinearity between factors (i.e., VIF < 5) (Tay 2017; Frost 2019), while the VIFs of population size and its interaction with population density were > 5 (Table 2) when the interaction was included into the model. However, the large VIF value for population size was caused by the introduction of interaction into the model, and the impact of interaction was a by-product that we

specified in the model. Therefore, the large VIF values of population size and interaction would not cause real multicollinearity problems (Frost 2019).

Results of path analysis show that both egg number and size had significant positive direct effects on sex ratio (Figure 2, Table 2). The significant positive effect of egg number on sex ratio may be because the number of both daughters and sons increased significantly with increasing egg number (coefficient=0.9518 and 0.4431, and t=95.16 and 15.06 for daughters and sons, respectively, P<0.0001), but the rate of increase (i.e., coefficient) was significantly greater for daughters than that for sons (non-overlapped 95% CLs of coefficients), entailing a strong female-biased sex ratio at the fecund patches. The significant positive direct effects of egg size on sex ratio suggest that *T. ludeni* females could adjust offspring sex ratio by altering the egg size and fertilizing larger eggs that develop to daughters. Similar findings have also been reported in other haplodiploid species, such as the spider mites *T. urticae* Koch (Macke *et al*. 2011, 2012) and thrips *Pezothrips kellyanus* (Bagnall) (Katlav *et al*. 2021).
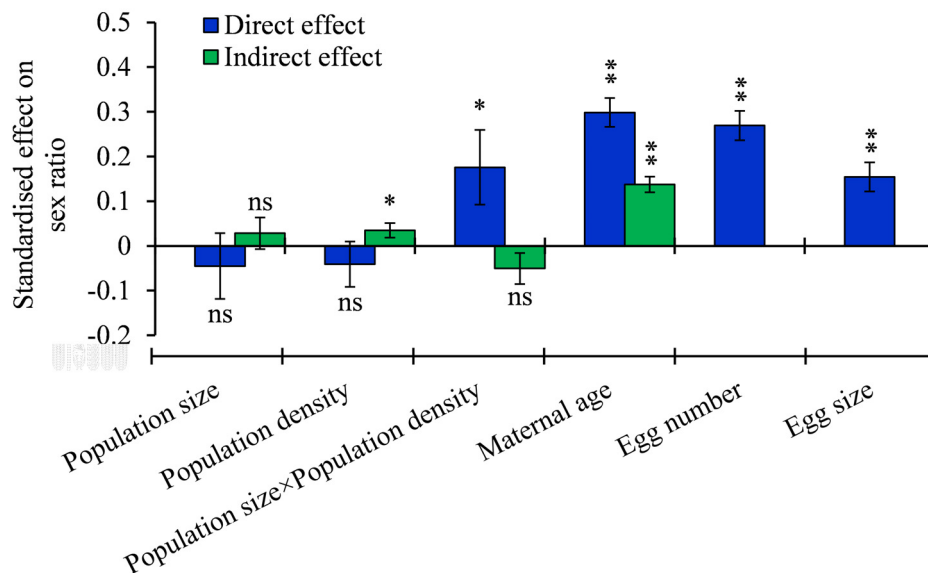

FIGURE 2. Standardized direct and indirect effects of social environments (population size and density and their interaction) and life history traits (maternal age, and egg number and size) on sex ratio in *Tetranychus ludeni*. For egg number and size, the indirect effect was not estimated. Standardised direct effect=-0.0452×PopuSize - 0.0411×PopuDens + 0.1758×PopuSize×PopuDens + 0.2983×MaternalAge + 0.2693×EggNo + 0.1544×EggSize, R^2=0.2729. ^{ns}, not significant; *, P < 0.05; **, P < 0.0001.

We further reveal that maternal age had both significant positive direct and indirect effects on sex ratio (Figure 2). The results suggest that the mated mothers did not experience any sperm depletion as single mating can lead to extremely female-biased sex ratios (Weerawansha *et al*. 2022b), and the aging mothers tend to increase fecundity as well as daughter production continuously, especially during their early life (Table 3; Weerawansha *et al*. 2020). Moreover, maternal age had significant positive effects on egg number (effect=0.4530, t=16.76, P<0.0001) and size (effect=0.1163, t=3.57, P=0.0004), leading to a significant positive indirect effect on sex ratio (Figure 2).

We provide strong evidence that social environments had weaker effects on *T. ludeni* sex allocation compared to the maternal age. First, population density significantly and indirectly affected the sex ratio probably due to its significant direct effect on egg number (effect=0.1471, t=2.90, P=0.0037) (Figure 2). Second, population size had no direct effect on sex ratio; in addition,

it exerted significant positive direct effect on egg number (effect=0.4953, t=8.56, P<0.0001) but significant negative effect on egg size (effect=-0.6960, t=-12.02, P<0.0001), which might have decreased the indirect effect of population size on sex ratio (Figure 2). Third, the interaction of population size and density had a significant positive direct effect on sex ratio; however, its significant contrary effects on egg number and size (i.e., effect=-0.5233 and 0.5093, and t=-7.34 and 7.13 for egg number and size, respectively, P<0.0001) might also counteract the indirect effect as did the population size (Figure 2). These results suggest that population size affecting *T. ludeni* sex allocation was implemented with population density and *vice versa* (Weerawansha *et al*. 2022a).

The full model equations of path analysis and multivariate linear regression that predicted the direct effects of different factors on sex ratio were similar (Figure 2, Table 2). Results of these models indicate that the direct effect of maternal age on sex ratio (effect≈0.30) was about 6.0, 7.0, and 1.6 times higher than that of population size, population density, and their interaction, respectively (Figure 2, Table 2). Moreover, results of the multivariate linear regression model show that maternal age had a much higher coefficient of determination (R^2=0.1848) than the social environments (R^2=0.0153) (Table 2), suggesting that maternal age alone explained more variance of offspring sex ratio compared to the social environments. The stepwise-selection method in the multivariate linear regression model further demonstrated an order of factors significantly affecting *T. ludeni* sex allocation: maternal age > egg number > egg size > interaction of population size and density (Table 2).

TABLE 2. Comparison of standardised direct effects of social environments [population size (PopuSize) and density (PopuDens) and their interaction (PopuSize×PopuDens)] and life history traits [maternal age (MaternalAge), egg number (EggNo), and egg size (EggSize)] on sex ratio in *Tetranychus ludeni*.

Factor	Effect (SE)	95% CLs	t	P	Partial R^2	VIF
Full model						
PopuSize	-0.0485 (0.0751)	-0.1960~0.0990	-0.65	0.5186	0.0030	6.37
PopuDens	-0.0367 (0.0522)	-0.1391~0.0657	-0.70	0.4816	0.0095	3.03
PopuSize×PopuDens	0.1805 (0.0868)	0.0102~0.3507	2.08	0.0378	0.0029	8.17
MaternalAge	0.3071 (0.0351)	0.2382~0.3760	8.75	<0.0001	0.1848	1.31
EggNo	0.2841 (0.0368)	0.2119~0.3563	7.72	<0.0001	0.0484	1.35
EggSize	0.1541 (0.0333)	0.0888~0.2195	4.63	<0.0001	0.0185	1.26
Final model						
MaternalAge	0.3084 (0.0347)	0.2401~0.3766	8.87	<0.0001	0.1874	1.29
EggNo	0.2800 (0.0357)	0.2099~0.3501	7.84	<0.0001	0.0434	1.27
EggSize	0.1574 (0.0303)	0.0978~0.2170	5.18	<0.0001	0.0232	1.05
PopuSize×PopuDens	0.1226 (0.0303)	0.0630~0.1821	4.04	<0.0001	0.0126	1.00

Full model: standardised direct effect=- 0.0485×PopuSize - 0.0367×PopuDens + 0.1805×PopuSize×PopuDens + 0.3071×MaternalAge + 0.2841×EggNo + 0.1541×EggSize, R^2=0.2671.
Final model: standardised direct effect=0.3084×MaternalAge + 0.2800×EggNo + 0.1574×EggSize + 0.1226×PopuSize×PopuDens, R^2=0.2666.

TABLE 3. Variations of egg number, egg size ($\times 10^6$ μm^3) and sex ratio (daughters%) of *Tetranychus ludeni* females over five days at different social environments of population size [PopuSize, female(s)] and density [PopuDens, female(s)/cm^2].

Parameter	PopuSize	PopuDens	Day 1	Day 2	Day 3	Day 4	Day 5
Egg no.	1	0.67	0.48 ± 0.13	2.76 ± 0.30	5.08 ± 0.41	3.72 ± 0.38	3.84 ± 0.55
		1	0.76 ± 0.22	2.33 ± 0.37	4.61 ± 0.48	4.33 ± 0.62	3.11 ± 0.53
		2	0.13 ± 0.07	2.58 ± 0.38	3.88 ± 0.33	5.21 ± 0.43	4.46 ± 0.44
	5	0.67	1.66 ± 0.14	3.50 ± 0.23	4.32 ± 0.33	5.08 ± 0.27	4.90 ± 0.28
		1	1.33 ± 0.15	2.91 ± 0.20	4.07 ± 0.31	4.88 ± 0.41	4.80 ± 0.37
		2	0.96 ± 0.24	2.86 ± 0.42	4.08 ± 0.48	4.22 ± 0.43	4.36 ± 0.48
	10	0.67	1.46 ± 0.08	5.64 ± 0.18	6.41 ± 0.37	6.81 ± 0.34	5.67 ± 0.36
		1	1.41 ± 0.10	4.20 ± 0.28	4.97 ± 0.45	4.88 ± 0.44	4.39 ± 0.45
		2	0.39 ± 0.18	2.64 ± 0.16	4.71 ± 0.24	3.93 ± 0.20	3.07 ± 0.14
Egg size	1	0.67	1.130 ± 0.041	1.160 ± 0.018	1.152 ± 0.017	1.156 ± 0.019	1.174 ± 0.021
		1	0.888 ± 0.027	1.049 ± 0.023	1.100 ± 0.014	1.118 ± 0.024	1.100 ± 0.016
		2	1.145 ± 0.031	1.101 ± 0.018	1.100 ± 0.014	1.106 ± 0.012	1.136 ± 0.018
	5	0.67	1.026 ± 0.010	1.022 ± 0.006	1.024 ± 0.010	1.010 ± 0.008	1.028 ± 0.009
		1	1.097 ± 0.017	1.090 ± 0.011	1.113 ± 0.011	1.097 ± 0.010	1.095 ± 0.011
		2	1.152 ± 0.032	1.131 ± 0.018	1.142 ± 0.015	1.148 ± 0.012	1.146 ± 0.010
	10	0.67	1.017 ± 0.007	1.014 ± 0.008	1.019 ± 0.009	1.015 ± 0.006	1.014 ± 0.006
		1	1.035 ± 0.014	1.060 ± 0.017	1.050 ± 0.014	1.070 ± 0.160	1.070 ± 0.022
		2	1.104 ± 0.041	1.099 ± 0.014	1.092 ± 0.012	1.102 ± 0.015	1.099 ± 0.014
Sex ratio	1	0.67	0.300 ± 0.153	0.759 ± 0.067	0.865 ± 0.044	0.948 ± 0.017	0.767 ± 0.067
		1	0.188 ± 0.132	0.637 ± 0.110	0.918 ± 0.033	0.887 ± 0.034	0.869 ± 0.062
		2	0.000 ± 0.000	0.567 ± 0.072	0.874 ± 0.035	0.872 ± 0.043	0.924 ± 0.023
	5	0.67	0.594 ± 0.031	0.772 ± 0.022	0.819 ± 0.012	0.803 ± 0.011	0.834 ± 0.013
		1	0.530 ± 0.047	0.822 ± 0.028	0.898 ± 0.016	0.859 ± 0.015	0.900 ± 0.014
		2	0.733 ± 0.053	0.782 ± 0.039	0.885 ± 0.024	0.865 ± 0.017	0.865 ± 0.015
	10	0.67	0.513 ± 0.037	0.836 ± 0.009	0.816 ± 0.014	0.814 ± 0.009	0.847 ± 0.012
		1	0.439 ± 0.045	0.939 ± 0.012	0.906 ± 0.011	0.894 ± 0.008	0.926 ± 0.011
		2	0.785 ± 0.138	0.864 ± 0.013	0.809 ± 0.023	0.878 ± 0.011	0.896 ± 0.008

In summary, we deciphered the direct and indirect effects and compared the direct effects of social environments and life history traits on sex allocation in *T. ludeni*. We demonstrate that the effects of life history traits on *T. ludeni* sex allocation outweigh that of social environments, and social environments influencing offspring sex ratios are implemented or mediated through life history traits, e.g., the egg number and size. However, a large amount of variance in sex ratio remains unexplained, indicating sex allocation in *T. ludeni* is more complex than expected. Therefore, further experiments integrating more life history traits and socio-environmental factors may help understand the complexity of sex allocation strategies in haplodiploid organisms.

Acknowledgements

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