

Figure S1. Photographs of male internal reproductive traits (testes, seminal vesicles, and each type of accessory gland) from SP treatment (A; upper figure) and AD treatment (B; upper figure) and female internal reproductive trait (the (a) length, (b) height, and (c) width of spermatheca) of SP treatment (A; lower figure) and AD treatment (B; lower figure) in *Euscepes postfasciatus*.

Supplemental Figure 2.

Effects of the larval diet on the size of spermatheca

Methods

To examine the difference in the size of the spermatheca, females were dissected in saline under a binocular microscope. The removed spermathecae were placed on a glass slide with a drop of distilled water (10 μ L) and covered with a cover glass. Under a stereomicroscope, the spermatheca's length, height, and width were measured using imaging software (see Fig. S1, SP treatment $n = 28$, AD treatment $n = 32$). The width of the spermatheca was defined as the length of the longest point from the sharp end on the left side to the right side of the organ, as shown in the photograph. Height was defined as the length of the longest point from the lower end to the upper end of the longest part on the right side. The width was defined as the length of the line starting at the bottom hollow and extending up to the point where it is taken vertically to the top.

Statistics

We used the GLM with a Gaussian distribution and identity link function to analyze the effects of diet during the larval stage of the female, and the effect of female body size on the size of the spermatheca. The significance level for all the tests was set at 5%. All analyses were conducted using the JMP software (ver. 14.3 [56], Cary, NC).

Results

Larval diet or body size did not significantly affected the size of the spermatheca (Fig. S2 (A) length: GLM, larval diet L-R $\chi^2 = 0.05$, $P = 0.81$; female body size L-R $\chi^2 = 1.56$, $P = 0.21$; larval diet \times female body size L-R $\chi^2 = 2.41$, $P = 0.12$ (B) height: larval diet L-R $\chi^2 = 0.29$, $P = 0.59$; female body size L-R $\chi^2 = 3.8$, $P = 0.051$; larval diet \times female body size; L-R $\chi^2 = 3.26$, $P = 0.07$ (C) width of spermatheca: larval diet L-R $\chi^2 = 0.04$, $P = 0.83$; female body size L-R $\chi^2 = 0.34$, $P = 0.56$; larval diet

× female body size L-R $\chi^2 = 3.28$, $P = 0.07$).

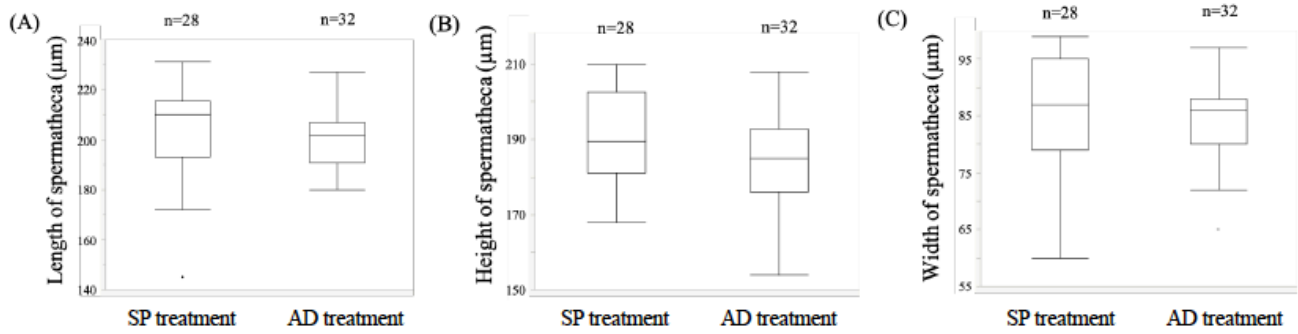


Figure S2. Comparison of the (A) length, (B) height, and (C) width of the spermatheca of different female treatments. Boxplots represent medians (thick lines), the first and third quartile ranges (box perimeters), minimum and maximum (whiskers), and outliers (small circles).

Table S1. (A) Ingredients, quantities and (B) nutrition component in the larval artificial diets (AD) and sweet potato tuber (SP).

† Contents of salt solution and vitamin mixture solution are referred to Okinawa Prefectural Protection Center (2018).

(A) Ingredients and quantities of AD

Ingredients	Quantity
Water	1 L
Agar (Ina-kanten®)	40 g
Cholesterol	800 mg
Potassium sorbate	1 g
Methyl p hydroxybenzoate	1 g
Salt mixture†	3 g
Powdered cellulose (KC flock®)	16 g
Powdered cellulose (KC flock®)	40 g
Soy bean meal (Esusan meat®)	24 g
AY-65 brewer's yeast	10 g
Vitamin mixture solution†	10 mL
Sweet potato powder	50 g
33% hydrochloric acid	20 mL

(B) Ingredients per 100g in AD and SP

	AD	SP
Protein	2.3 g	1.2 g
Fat	0.2 g	0.2 g

Carbohydrate	7.3 g	31.9 g
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References: Ohishi et al (2018) and Standards Tables of Food Composition in Japan - 2020- (Eighth Revised Edition).

Table S2. Estimated size of proteins (kDa) including in in the testes-seminal vesicles complex (TSVC), and accessory gland B (AGB) and accessory gland C (AGC) of *E. postfasciatus*. AGB No. 25, surrounded by dash lines, is an SP specific protein.

TSVC			AGB			AGC		
No.	Relative distance from the top	Estimated size (kDa)	No.	Relative distance from the top	Estimated size (kDa)	No.	Relative distance from the top	Estimated size (kDa)
1	168	262.184992	1	166	266.525438	1	166	266.525438
2	188	224.717266	2	194	215.243816	2	186	228.036627
3	330	103.90208	3	292	122.875972	3	284	127.646059
4	350	95.8494368	4	332	103.044909	4	324	106.549066
5	374	87.5185044	5	358	92.9251401	5	348	96.6054684
6	404	78.733218	6	376	86.8809015	6	368	89.4807251
7	420	74.6503893	7	410	77.1578623	7	402	79.2707442
8	440	70.0379192	8	428	72.744045	8	420	74.6503893
9	524	55.1192957	9	446	68.7493763	9	436	70.9203512
10	566	49.590162	10	498	59.102442	10	488	60.7691663
11	590	46.8455936	11	532	53.9861064	11	522	55.409036
12	638	42.082129	12	586	47.2845459	12	576	48.4136281
13	652	40.8482448	13	648	41.1943373	13	632	42.6308252
14	674	39.0314006	14	680	38.5600097	14	664	39.8395494
15	908	25.9400707	15	784	31.7248467	15	774	32.2881377
16	1040	21.5354597	16	878	27.1628836	16	866	27.6802383
17	1376	14.6710427	17	908	25.9400707	17	906	26.0186102
18	1426	13.9704046	18	972	23.6274256	18	952	24.3105955
19	1492	13.1301533	19	1018	22.1760734	19	1004	22.6011186
			20	1050	21.2547659	20	1042	21.4788098
			21	1110	19.6955925	21	1098	19.9913004
			22	1144	18.8975183	22	1130	19.2192443
			23	1186	17.9860893	23	1174	18.2386166
			24	1328	15.4028928	24	1318	15.5633408
			25	1350	15.0598018	25	1370	14.7592047
			26	1378	14.6418575	26	1404	14.271399
			27	1438	13.8108186	27	1430	13.9168564