Supplementary methods

(a) Fly stock maintenance

The Dahomey strain was collected in Benin (Africa) in the 1970s and has since then been kept as a cage population at large population size with overlapping generations. The population is fed weekly with three glass bottles each containing 70 mL of the sugar-yeast food. The Dahomey population has been frequently used in studies investigating Sfps and SP specifically e.g. [1,2]. Both the Innisfail and the Melbourne strains originate from Australia and were collected in Innisfail, Queensland (latitude 17.52°S) and Melbourne, Victoria (latitude 37.77 °S) in 2008 [3]. The sampling points represent the two opposing ends of a transect on the eastern coast of Australia with different climates: temperate (Melbourne) and tropical (Innisfail). We kept both strains at smaller population sizes in glass bottles supplemented with 70 mL sugar-yeast food for five to six years prior to conducting experiments. Newly eclosed adults were transferred to fresh food at day 14 of the generation cycle. However, bottles were not strictly cleared of adults and occasionally combined across generations, thus generations could in principle overlap.

(b) Detailed experimental procedure for single mating experiments

All individuals were virgin and three to four days post-eclosion at the start of each experiment. We always added females individually without anaesthesia to vials containing one male and continuously observed pairs for two hours for a mating to occur. Males were discarded immediately after the mating ended. Successfully mated females remained in their vials to oviposit. After 24 hours, we transferred each female to a fresh vial containing one Dahomey male and recorded the number of females remating within one hour. We counted number of eggs laid in the vacated vials and later also counted the number of eclosing adult offspring. We recorded mating frequency and remating propensity as a binary response (mating occurred or not). In all three experiments, we set up 40 pairs per mating.

(c) Calculation of the intrinsic population growth rate r

Lifetime adult offspring production and survivorship data were used to calculate the intrinsic rate of population increase (r) as an index of fitness [1,4], where r is derived from the Euler equation:

$$1 = \sum_{x=0}^{k} e^{-rx}l(x)b(x)$$

Here x is female age in days, l(x) the probability to survive to age x, b(x) the number of adult offspring produced at age x, and k the age at last egg laying.
(d) Detailed experimental procedure for injection of synthetic SP into virgin females

Synthetic SP was kindly provided by T. Aigaki at Tokyo Metropolitan University, Japan and generated as described in [5]. Synthetic SP was diluted in Drosophila Ringer’s solution [6] to different concentrations to obtain dose response curves. As our populations were unresponsive to injections of low concentrations between 0 and 5 pmol (Fig. S1), a range previously shown to induce a PMR [7], we increased the concentrations to 5, 6, 7, 8, 9, and 10 pmol. For each of the six concentrations we injected 21 virgin females from each population with 0.05 µl of synthetic SP with a Nanoject II (Drummond Scientific Company). As a control, 21 virgin females from each population were injected with Drosophila Ringer’s solution.

(e) Detailed RNA isolation and qPCR protocol

Virgin four-day old females from each population (Dahomey, Innisfail and Melbourne) were snap frozen in liquid nitrogen and stored at -80 °C until dissection. Abdomens were separated from the thorax on ice using a scalpel and stored in pools of ten at -80 °C until RNA isolation. We collected five replicate samples for each population. Samples were homogenised with stainless steel beads (Qiagen) in a mill (Retsch) followed by RNA isolation using the RNeasy Minikit (Qiagen) according to the manufacturer’s protocol. 200 ng RNA per sample were reverse transcribed using the Revert Aid cDNA synthesis kit with oligodT primers (Thermo Scientific). We used the undiluted cDNA to quantify expression of SPR relative to two reference genes, ribosomal protein L13a (RpL13a) and elongation factor 1a48D (Ef1a48D) on a LightCycler 480 (Roche) using KAPA SYBR Fast qPCR Mastermix (peqlab) in a 15 µl reaction volume (7.5 µl SYBR Fast, 0.4 µl forward primer, 0.4 µl reverse primer, 4.7 µl water and 2 µl cDNA template; cycle conditions: 3 min pre-incubation at 95 °C, followed by 40 amplification cycles each consisting of 15 s at 95 °C and 1 min at 60 °C, followed by heating up to 97 °C and continuously measuring the fluorescence to obtain melting curves). See Table S1 for primer sequences. Each sample was run in duplicate and non-template controls were included for all three primers.

Primer efficiencies for all primers were determined using fivefold dilution series (1, 1:5, 1:25, 1:125, 1:625) in duplicates and efficiency calculated as $E = 10^{-1/\text{slope}} - 1$ (Table S1). The arithmetic mean for crossing point (Cp) values of the technical replicates were obtained from the LightCycler software with the second derivative maximum method and used to calculate relative expression $R$ after Pfaffl [8].

$$ R = \frac{E^{\Delta Cp_{\text{target}} \text{ (calibrator-sample)}}}{\text{GM} \left( E^{\Delta Cp_{\text{reference}} \text{ (calibrator-sample)}} \right)} $$

Where $E$ is the efficiency of the respective primer pair and GM the geometrical mean of the two reference genes. The mean Cp of the five Dahomey samples was used as the calibrator, thereby we set SPR expression to 1 in the Dahomey samples and SPR expression in Innisfail and Melbourne is calculated relative to expression in the Dahomey population.

Table S1: Sequence and efficiencies for gene specific primers used in qPCR

| Gene  | Sequence (5’-3’) | Efficiency | Reference |
|-------|-----------------|------------|-----------|
| RpL13a Forward: AAGGCAGTCGGAGGATGATCC | 0.92 | [9] |
| Reverse: CGACGCTTGCAGGTAGGCGA |
| Ef1a48D Forward: TCATTACGTTATTCCAGACCATAG | 0.89 | [10] |
| Reverse: TCGATGGTACGCTTGTGAT |
| SPR Forward: TGCTGTCGTATGCTTCCCTCC | 0.95 | This study |
| Reverse: CGCATTAGCCATTTCCCATAGC |
(f) Detailed PCR protocol:

We isolated genomic DNA from single flies by homogenising the whole fly in 50 µl squishing buffer (10 mM Tris-HCl, 1 mM EDTA, 25 mM NaCl with freshly added Proteinase K (20 µl of 10 mg/ml solution in PBS per 1 ml buffer)) and subsequent incubation for ten minutes at 37 °C followed by enzyme inactivation at 95 °C for three minutes. We centrifuged samples briefly to separate the debris and transferred the supernatant containing the gDNA to a fresh tube. We used 1 µl gDNA to amplify SP and SPR in a PCR (0.2 mM dNTPs, 2.5 mM MgCl₂, 0.4 mM each primer and 0.5 U GoTaq Flexi (Promega) in a 25 µl reaction volume) with gene specific primers (Table S2). The amplification conditions were as follows: 3 minutes at 95 °C, 32 cycles of 94 °C for 20 seconds, 55°C for 20 seconds and 72 °C for 1 minute followed by 7 minutes at 72 °C.

Table S2: Sequence of primers used to amplify and sequence SP and SPR

| Gene    | Forward sequence (5’→3’) | Reverse sequence (5’→3’) | Product size |
|---------|--------------------------|--------------------------|--------------|
| SP      | AGTGTGAAAAACCTTAACCGTGTC | GTCCCCCAAATAAGGCAATAAG  | 493 bp       |
| SPR exon 6 | TGATACCGCCTAATTCCTGTCTT | GCTCCTCGGTGATGATGTC    | 989 bp       |

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Figure S1. Post-mating responses by females after a single mating to coevolved males from their own population. 24 hours after a first mating we measured (a) the mean number of eggs (± SE) produced per female and (b) the proportion of females remating (± SE) with Dahomey males within 1 hour. Different letters indicate significant contrasts (\(P < 0.05\)) between populations based on Tukey’s post hoc test. Flies were from three different wild type populations: Dahomey, Innisfail and Melbourne.
Figure S2. Post-mating responses of DGRP776 females after a single mating to males from three different wild type populations: Dahomey, Innisfail and Melbourne. 24 hours after a first mating we measured (a) the mean number of eggs (± SE) produced per female and (b) the proportion of females remating (± SE) with Dahomey males within 1 hour.
Figure S3: Female fitness measured as the intrinsic rate of population growth $r$ when exposed continuously to $SP^+$ (grey bars) or $SP^0$ (white bars) males. Females were from three different wild type populations: Dahomey, Innisfail and Melbourne. Repeated receipt of SP increased the index of fitness for Innisfail and Melbourne females with the effect being stronger in Innisfail females while the index of fitness was similar for Dahomey females with both male genotypes.
Figure S4: Female post-mating responses in different wild types (Dahomey: yellow diamonds, Innisfail: blue squares, Melbourne: brown triangles) to low concentrations (0 – 5 pmol) of synthetic SP at 6 hours post injection. (a) Proportion of females that laid eggs in response to SP injection and (b) proportion of females that mated with a Dahomey male within one hour. Points represent means (± SE) calculated from raw data.
Figure S5: Female post-mating responses in three wild types (Dahomey: yellow diamonds, Innisfail: blue squares, Melbourne: brown triangles) in response to injection of synthetic SP at different concentrations 6 hours post injection. (a) Proportion of females that laid eggs and (b) proportion of females that mated with a Dahomey male within one hour. Points represent means (± SE) calculated from raw data, lines are calculated from the statistical model (long dashed line: Dahomey, dashed line: Innisfail, solid line: Melbourne).
Figure S6: Mean expression (± SE) of SPR in the abdomen of Innisfail and Melbourne females relative to SPR expression in the abdomen of Dahomey females (set to 0). Dashed lines indicate upper and lower SEs for Dahomey.