

Figure S1 Expression of the GFP-tagged SP proteins in flies. A series of the GFP tagged SPs were expressed in eye imaginal disc using GMR-GAL4. Three 4-day-old female flies were subjected to immunoblot analysis using an anti-GFP antibody. The GFP-tagged SP proteins (G-SPn, G-SP and G-SPc) or GFP alone (sGFP) were expressed as immunoreactive products with expected sizes.

A

	Signal Peptides	Exon/Intron Boundary	
<i>D.mel</i>	MKT-LALFLVLVC--VLGLV-QA--WEWPWN---RKPTKFP I P S P N P R	↓	--DKWCRLNLGPAWGGR-C 55
<i>D.sim</i>	MKT-LSLFLVLVC--LLGLV-QS--WEWPWN---RKPTKYPI P S P N P R		--DKWCRLNLGPAWGGR-C 55
<i>D.sec</i>	MKT-LSVFLVLVC--LLGLV-QS--WEWPWN---RQPTRYPI P S P N P R		--DKWCRLNLGPAWGGR-C 55
<i>D.yak</i>	MNT-VALLLVLVC--IVSLV-QS--WTWPWQK--KKP-KFP I P S P N P R		--DKWCRLNLGPGWGGR-C 55
<i>D.ere</i>	MKA-VSLLLVLVC--IVGLV-QS--WTWPWQK--KPEVKFP I P S P N P R		--DKWCRLNLGPGWGGR-C 56
<i>D.ana</i>	MKN-FNILLLVAYAIMVAMV-SS--ERMTWLKTLSPWRPMPMSAR		--HKWCRLNLGPLWGGR-C 60
<i>D.pse</i>	MKVATSAMLLML--VEAAVGVPA-WGRMTS---RRPT--PKQSQAQF		--QKWCRLNFGPAWGGRG-C 57
<i>D.per</i>	MKVATSAMLLML--VEAAVGVPA-WGRMTS---RRPT--PKQSQAQF		--QKWCRLNFGPAWGGRG-C 57
<i>D.wil</i>	MQAPISILLLL----VLAIVSQSMA-----NPNPERGGDKG		KWCRLNLGPAWGGR-C 47
<i>D.vir</i>	MQATFSIIFIL----LSILCCSRG-----EYKTTKWPRYPNKWCRLNYPYLGGR-C 47		

B

<i>D.mel</i>	CCAAGCCCAATCCTC P**S**P**N**P**R	gtaagtgtt - 45 bp - tctttcgtag	-----GTGATAAGTGGTGCCGT -----**D**K**W**C**R**
<i>D.sim</i>	CCAAGCCCAATCCTC P**S**P**N**P**R	gtaagtctta - 54 bp - tctttcgcag	-----GTGACAAGTGGTGCCGT -----**D**K**W**C**R**
<i>D.sec</i>	CCAAGCCCAATCCTC P**S**P**N**P**R	gtaagtttta - 45 bp - tctttcgcag	-----GTGACAAGTGGTGCCGT -----**D**K**W**C**R**
<i>D.yak</i>	CCAAGCCCAATCCTC P**S**P**N**P**R	gtaagtgtga - 32 bp - tcttttgcag	-----GTGATAAGTGGTGTCGT -----**D**K**W**C**R**
<i>D.ere</i>	CCAAGCCGAATCCTC P**S**P**N**P**R	gtaagttttt - 31 bp - tctttcgcag	-----GTGATAAGTGGTGCCGT -----**D**K**W**C**R**
<i>D.ana</i>	CCTATGACTTCGGCTC P**M**T**S**A**R	gtaactgtct - 42 bp - ttttttcaag	-----GTCACAAGTGGTGCCGA -----**H**K**W**C**R**
<i>D.pse</i>	CAAAGCCAAGCACAAT S**Q**Q**A**Q**F	gtaagtaaat - 54 bp - tcgcctacag	-----TTCAGAAATGGTGTAGA -----**Q**K**W**C**R**
<i>D.per</i>	CAAAGCCAAGCACAAT S**Q**Q**A**Q**F	gtaagtaaat - 54 bp - tcgccaacag	-----TTCAGAAATGGTGTAGA -----**Q**K**W**C**R**
<i>D.wil</i>	AATCCTGAAAGAGGAG N**P**E**R**G**G	gtatgtaaac - 41 bp - ccgattcaag	GTGATAAAGCAAATGGTGTCGT **D**K**G**K**W**C**R**
<i>D.vir</i>	ACGACCAAATGGCCAC T**T**K**W**P**R	gtaagtaaac - 52 bp - attgccttag	GTTATCCCAATAAATGGTGTCGT **Y**P**N**K**W**C**R**

Conserved Domain

Figure S2 Comparison of amino acid sequences and gene structures of SP orthologs. (A) Comparison of amino acid sequences of SP orthologs. Black and gray boxes indicate highly conserved amino acid residues. A black bar and an arrow indicate signal peptides and exon/intron boundaries, respectively. Aligned sequences were inspected and manually adjusted to maximize homology. (B) Schematic presentation of exon/intron boundaries of SP orthologs. Boxed and unboxed regions indicate exons and introns, respectively. Putative amino acid residues were shown below DNA sequences. Gray boxes indicated splicing consensus sequences.



Figure S3 G-SP-binding ability to female reproductive organs of various *Drosophila* species. Female reproductive organs from indicated *Drosophila* species were co-incubated with head homogenates of *GMR>G-SP* flies.

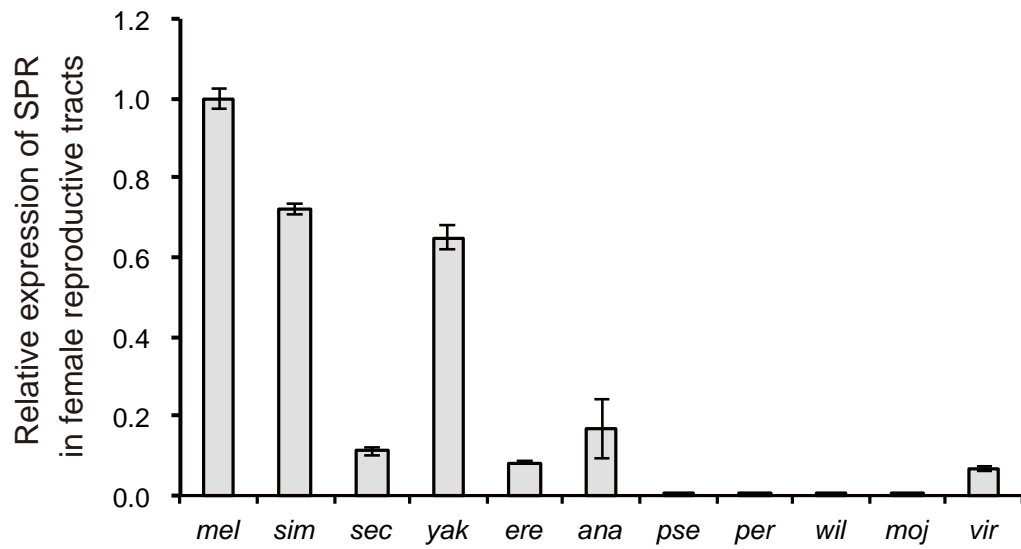


Figure S4 Expression levels of SPR orthologs in the female reproductive organs of various *Drosophila* species. SPR was highly expressed in the *D. melanogaster* species group, but not in distant species, except for *D. virilis*.

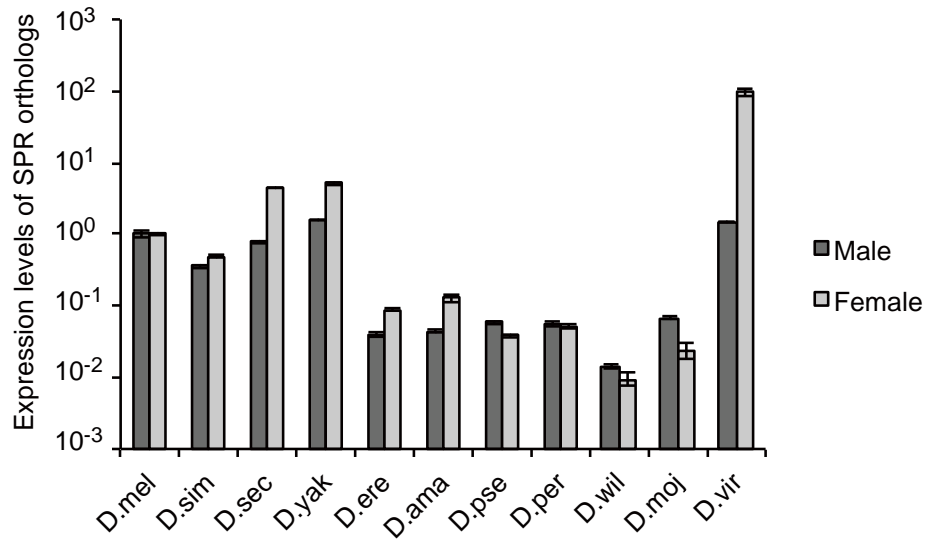


Figure S5 Expression levels of SPR orthologs in whole-body of various *Drosophila* species. SPR was highly expressed in both sexes of all of *Drosophila* species we tested.

File S1

Visualizing transfer of G-SP from male to female *in vivo*.

The G-SP-expressing transgenic male (left) was mated with a wild-type female (right).

Available for download as a .mov file at

www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.177550/-/DC1