fruitless regulates aggression and dominance in *Drosophila*

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When competing for resources, two *Drosophila melanogaster* flies of the same sex fight each other. Males and females fight with distinctly different styles, and males but not females establish dominance relationships. Here we show that sex-specific splicing of the *fruitless* gene plays a critical role in determining who and how a fly fights, and whether a dominance relationship forms.

Aggression involves a set of stereotyped behavioral patterns that are triggered by competition over food, territory or mates. Like any instinct, aggression must ultimately have a strong genetic basis, yet the specific genes that program aggressive behaviors into the nervous system are unknown. Recently, *D. melanogaster* has emerged as a powerful model system to study the genetic and neural basis of aggression[1–5]. Under the appropriate conditions, pairs of male or female flies will fight each other, displaying a distinctive set of aggressive behaviors (**Supplementary Videos 1** and **2** online). Some of these behavioral components are common to both male and female fights, such as low-intensity 'fencing.' Other components, particularly those of higher intensity, are much more frequent in one sex than the other. For example, 'lunging' and 'boxing' are mostly seen in male fights, whereas 'shoving' and 'head-butting' are characteristic of female fights. Another distinguishing feature of male and female fights is that males form dominance relationships, whereas females do not[5]. That is, in a typical male fight, the male that wins the initial encounters wins almost all subsequent encounters. In contrast, in a female fight, the opponents tend to win alternately and share the resource.

We speculated that the *fruitless* (*fru*) gene might be involved in specifying these sex differences in aggression and dominance. This inference was based on *fru*'s critical role in another sex-specific social behavior, male courtship[6,7], as well as on an earlier report of anomalous interactions in *fru* mutant males[8] that were subsequently found to be characteristic of normal female fights[5]. The *fru* gene produces multiple transcripts, all of which are thought to encode zinc-finger transcription factors[9,10]. Transcripts from the distal P1 promoter are sex-specifically spliced, resulting in male-specific mRNAs that encode full-length Fru proteins (Fru[M]) and female-specific mRNAs that are evidently not translated. We previously generated alleles of *fru* that are constitutively spliced in either the male (*fru[M]*) or female (*fru[F]*) mode, irrespective of the sex of the fly[11]. An additional control allele (*fru[C]*) is subject to normal sex-specific splicing.

We used these *fru* splicing alleles—*fru[C]*, *fru[M]*, and *fru[F]*—to study the role of *fru* in aggression. We concentrated on the five pairwise combinations of these flies that do not result in a high rate of courtship: two *fru[C]* males, two *fru[C]* females, two *fru[F]* males, a *fru[F]* male and a *fru[C]* female, and a *fru[F]* male and a *fru[M]* female. Specifically, we addressed the following questions: (i) Is female splicing necessary and sufficient to specify female-style aggressive behavior? (ii) Is male splicing necessary and sufficient for male-style aggression, as it is for male courtship? (iii) How fixed is a fly's fighting pattern—can it adjust its strategy to that of its opponent? (iv) Is *fru* involved in the formation of dominance relationships?

To assay aggression, we placed pairs of flies in an observation chamber designed to promote aggressive interactions[4]. This chamber contains an elevated and illuminated food plate, a desired resource for which the two flies compete. We monitored the behavior of each fly for the first 30 min after they both appeared on the food plate, recording the total number of interactions, and for each fly whether the interaction involved aggression, courtship or a mixture of the two (**Fig. 1a**). Aggressive encounters were further scored for specific mid- and high-intensity components (**Fig. 1b**).
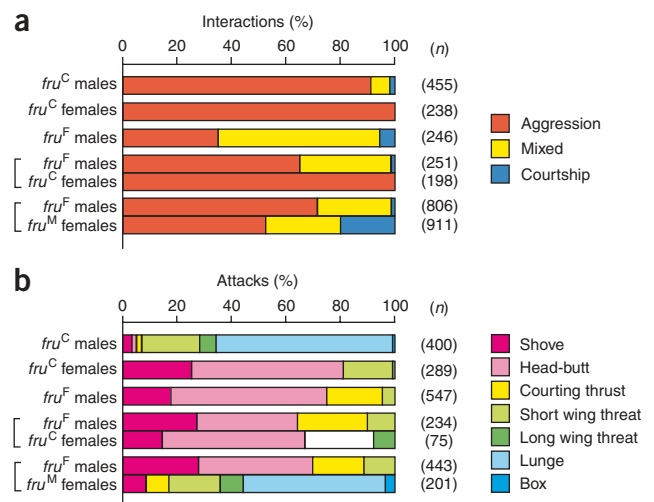

Figure 1 Patterns of aggression in *fru* splicing mutants. (**a**) Percentage of aggressive, courting or mixed interactions from each fly, in assays involving either a pair of *fru[C]* males, a pair of *fru[C]* females, a pair of *fru[F]* males, a *fru[F]* male and a *fru[C]* female, or a *fru[F]* male and a *fru[M]* female. For the symmetric fights, data for the two flies are pooled. *n*, number of interactions observed (in 10, 10, 8, 9, and 24 movies respectively). (**b**) Percentage of selected mid- and high-intensity attacks observed during aggressive interactions, for each of the five combinations as in **a**.

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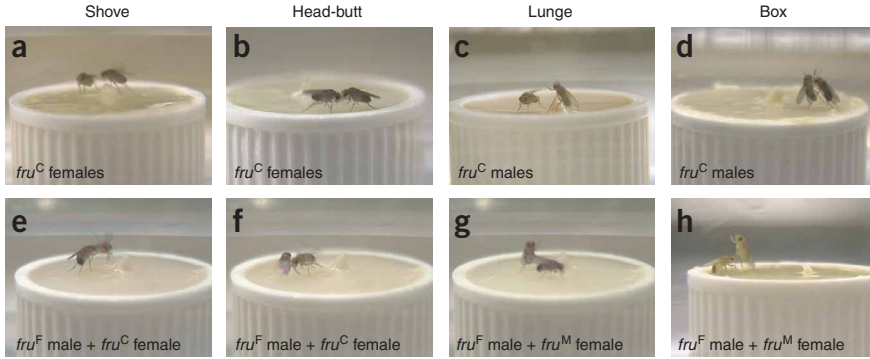
Figure 2 Selected aggression scenes. Selected scenes from aggression assays involving (**a,b**) a pair of *fru*^C females, (**c,d**) a pair of *fru*^C males, (**e,f**) a *fru*^F male and a *fru*^C female, and (**g,h**) a *fru*^F male and a *fru*^M female. (**a,e**) A shove, performed by a *fru*^C female, **a**, and a *fru*^F male, **e**. (**b,f**) A head-butt, performed by a *fru*^C female, **b**, and a *fru*^F male, **f**. (**c,g**) A lunge, performed by a *fru*^C male, **c**, and a *fru*^M female, **g**. (**d,h**) Boxing, performed by a *fru*^C male, **d**, and a *fru*^M female, **h**. Note that when one *fru*^C male rises to box, a *fru*^C male opponent normally does likewise, **d**. However, when a *fru*^M female rises to box, a *fru*^F male opponent usually retreats or counters with a head-butt or shove, **h**.

We began by asking who *fru*^F males fight, and how they fight. These males interacted equally often with each of the three types of opponent we offered them: another *fru*^F male (30.9 ± 5.2 interactions, $n = 8$), a *fru*^C female (27.9 ± 4.9, $n = 9$) or a *fru*^M female (40.7 ± 6.5, $n = 24$) ($P = 0.40$, one-way ANOVA). The nature of these interactions was also similar in fights with *fru*^M females and *fru*^C females, with ~2/3 of all interactions being classified as aggression and ~1/3 as mixed aggression and courtship (**Fig. 1a**). Pure courtship toward either type of female was rare (<1.5% of all interactions), consistent with the results obtained using courtship rather than aggression chambers[11]. Clearly, *fru*^F males are much more likely to fight females than to court them. Their attitude toward other *fru*^F males was more ambivalent. Interactions between pairs of *fru*^F males were still mostly aggressive, but aggression was more often mixed with courtship than when *fru*^F males fought either *fru*^C or *fru*^M females.

Despite the fact that *fru*^F males are slightly less likely to fight other *fru*^F males than either *fru*^M females or *fru*^C females, the pattern of aggression was similar in all three cases (**Fig. 1b**, **Supplementary**

Videos 3–5 online). Hence, the identity of the opponent may influence the inclination or *fru*^F males to fight, but not the manner in which they fight. The prominent modes of attack in all of these fights were the head-butt and the shove, as well as a component newly described here that we call the 'courting thrust' (which resembles the aggressive shove or head-butt but occurs during a mixed interaction that involves courtship as well as aggression). We never observed a *fru*^F male to lunge or box in any of the three pairings. This pattern of *fru*^F male aggression closely matches that of control *fru*^C female fights (**Figs. 1b** and **2a,b,e,f**; **Supplementary Video 2**), with the exception that *fru*^C female interactions were exclusively aggressive and so the courting thrust was omitted. In summary, *fru*^F males were more inclined to fight females than to court them, and also somewhat more inclined to fight females than males (**Fig. 1**). Regardless of the sex of their opponent, when *fru*^F males fought, they fought like females.

How do *fru*^M females behave? In pairings with other females, they courted, whether in a courtship chamber[11] or an aggression chamber (data not shown). Also, in pairings with normal males, *fru*^M females are vigorously courted, and so their interactions with these males primarily involve courtship rejection rather than aggression[11]. Hence, the only combination in which we could elicit robust aggression from *fru*^M females was by pairing them with *fru*^F males. The majority of interactions we observed in such pairs were indeed exclusively aggressive, with the remainder being either courtship or mixed (from the perspective of the *fru*^M female; **Fig. 1a**). The pattern of aggression shown by these *fru*^M females was indistinguishable from that shown by control *fru*^C males ($P = 0.10$, $\chi^2{}_2$-test, **Figs. 1b** and **2c,d,g,h**; **Supplementary Videos 5** and **6**). In both cases, ~60% of mid- and high-intensity attacks involved lunges, which were never performed by *fru*^F males or *fru*^C females in any of their fights. Thus, *fru*^M females tend to court females[11] and fight males, thereby reversing the normal female sexual

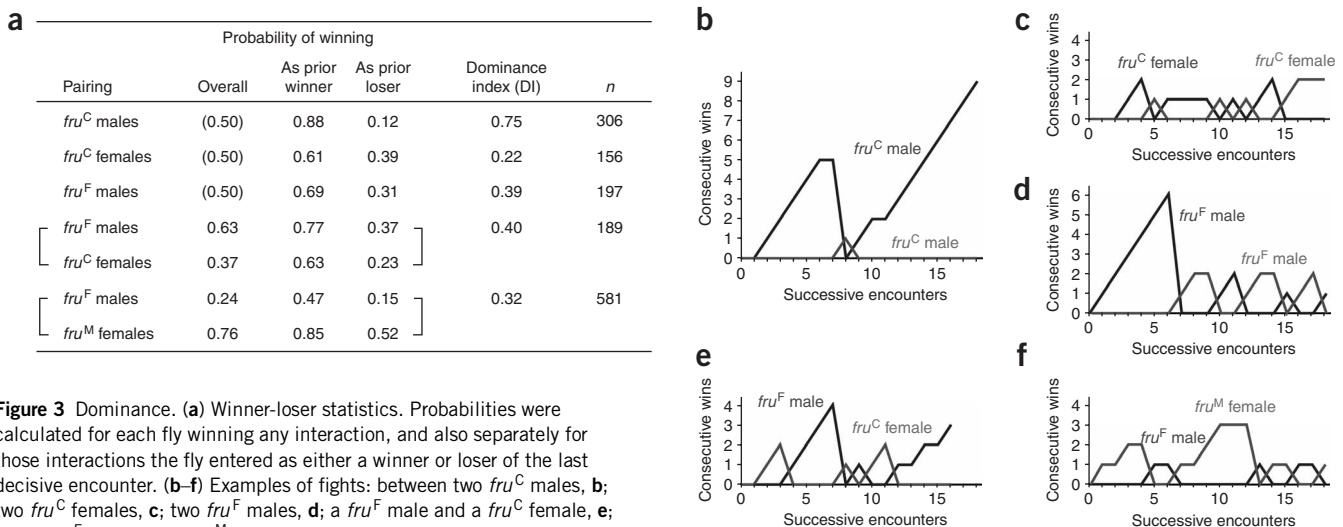| Pairing | Probability of winning | | | Dominance index (DI) | *n* |
	Overall	As prior winner	As prior loser		
fru^C males	(0.50)	0.88	0.12	0.75	306
fru^C females	(0.50)	0.61	0.39	0.22	156
fru^F males	(0.50)	0.69	0.31	0.39	197
fru^F males	0.63	0.77	0.37	0.40	189
fru^C females	0.37	0.63	0.23		
fru^F males	0.24	0.47	0.15	0.32	581
fru^M females	0.76	0.85	0.52		

Figure 3 Dominance. (**a**) Winner-loser statistics. Probabilities were calculated for each fly winning any interaction, and also separately for those interactions the fly entered as either a winner or loser of the last decisive encounter. (**b–f**) Examples of fights: between two *fru*^C males, **b**; two *fru*^C females, **c**; two *fru*^F males, **d**; a *fru*^F male and a *fru*^C female, **e**; and a *fru*^F male and a *fru*^M female, **f**. The number of consecutive wins for each fly is shown. Note that not all encounters are decisive. A clear dominance relationship is evident in the *fru*^C male fights, **b**, but not in any of the other combinations, even though the *fru*^F male scores more wins overall in the fight with the *fru*^C female, **e**, and fewer wins against the *fru*^M female, **f**.

orientation. Whether courting or fighting, they behave like males. Thus, just as female splicing of fru is necessary and sufficient for female-style aggression, male splicing is necessary and sufficient for male-style aggression.

We also note the unique character of fights between fru^F males and fru^M females. In such pairings, the males fight like females while the females fight like males (**Figs. 1b** and **2g,h**). Their styles are totally mismatched, as evidenced for example in scenes in which the fru^M female boxes or lunges while the fru^F male head-butts. Yet despite this disparity of styles, the behavior of the fru^M females was quantitatively indistinguishable from that of fru^C males in control male fights ($P = 0.10$, χ^2_2-test), and the behavior of fru^F males was barely distinguishable from that of fru^F males in style-matched fights with fru^C females (**Fig. 1b**, $P = 0.01$, χ^2_3-test). Thus, how the opponent fights has little influence on how a fru^F male or a fru^M female fights. Their patterns of aggression seem to be fixed, at least within the context of an single fight.

To assess whether fru might be involved in the formation of dominance relationships, we determined the winner and loser of each decisive encounter for all five pairings. The winner was defined as the fly that escalated to the highest intensity component or chased the other fly away. In control fru^C male fights, the same fly usually won each successive encounter, whereas in fru^C female fights the winner of one encounter had only slightly better than even chances of winning the next (**Fig. 3a–c**). To compare the degree of dominance in such fights, we defined a dominance index (DI) as the difference the outcome of one encounter makes in each fly's chances of winning their next encounter. Specifically, DI = P(winning as prior winner) – P(winning as prior loser). Note that the DI is the same for both flies in a fight, as one fly's win is the other's loss. If the outcome of one encounter has no influence on the next, DI = 0. If winning one encounter guarantees winning the next, DI = 1, and if it guarantees losing, DI = –1. For the fru^C male fights, DI = 0.75, and for fru^C female fights DI = 0.22 (**Fig. 3a**). This pattern accords with the previous report that males but not females establish strong dominance relationships[5].

For fights between two fru^F males, the winner of one encounter also had only slightly better than even chances of winning the next, and for these fights DI = 0.39. This value is marginally higher than in control fru^C female fights ($P = 0.024$, Monte Carlo simulation; see **Supplementary Methods** online) but still significantly lower than in fru^C male fights ($P < 0.0001$; **Fig. 3a,d**). We thus conclude that fru^F males, like normal females, do not establish strong dominance relationships.

The mixed fights between fru^F males and either fru^C females or fru^M females have an inherent bias, in that fru^F males generally beat fru^C females (winning 63% of encounters, $n = 189$), but lost to fru^M females (winning only 24% of encounters, $n = 581$). Nevertheless, in each case, the fly that won an encounter improved its chances of winning the next, indicating that some degree of dominance is established (**Fig. 3a**). However, the DI for fru^F males fighting either fru^C females (0.40) or fru^M females (0.32) was no different from that observed in fights between two fru^F males (0.39, $P = 0.80$ and $P = 0.18$, respectively; **Fig. 3a,e,f**). Thus, although these mixed-sex fights are biased, dom-

inance relationships are no more likely to form than in the evenly matched control fru^F male fights. Moreover, fru^M and fru^C females do not differ in their ability to form dominance relationships in fights with fru^F males. This might indicate that, although necessary, fru^M is not sufficient for the formation of strong dominance relationships. Alternatively, and in our view more probably, it may reflect the fact that both flies must participate in the formation of a dominance relationship. Distinguishing between these two possibilities will require finding a way to induce two fru^M females to fight rather than court each other.

In conclusion, our data indicate that aggressive behaviors are hardwired into the fly's nervous system and that fru plays a critical role in establishing both sex-specific patterns of aggression and the ability to form dominance relationships. In light of the well established role of fru in courtship behavior, these finding suggest that, genetically, aggression and mating are closely intertwined in *Drosophila*, as appears to be the case also in mammals[12,13]. An important and fascinating task for the future is to try to disentangle these two opposing instincts by identifying the genes and the neural circuits unique to each.

Note: Supplementary information is available on the Nature Neuroscience website.

AUTHOR CONTRIBUTIONS
E.V. conducted the experiments together with S.P.N. in the laboratory of E.A.K.; E.D. generated various fly stocks; E.V. and B.J.D. analyzed the data; and B.J.D. wrote the manuscript with contributions from all other authors.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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