Influence of the **White** Locus on the Courtship Behavior of *Drosophila* Males

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**Abstract**

Since its discovery by Morgan, the *Drosophila white* gene has become one of the most intensely studied genes and has been widely used as a genetic marker. Earlier reports that over- and misexpression of White protein in *Drosophila* males leads to male-male courtship implicated *white* in courtship control. While previous studies suggested that it is the mislocalization of White protein within cells that causes the courtship phenotype, we demonstrate here that also the lack of extra-retinal White can cause very similar behavioral changes. Moreover, we provide evidence that the lack of White function increases the sexual arousal of males in general, of which the enhanced male-male courtship might be an indirect effect. We further show that *white* mutant flies are not only optomotor blind but also dazzled by the overflow of light in daylight. Implications of these findings for the proper interpretation of behavioral studies with *white* mutant flies are discussed.

**Introduction**

The vinegar fly *Drosophila melanogaster* serves as an excellent model system to study the control of innate behaviors by the nervous system. The mating behavior of *Drosophila* consists of well-defined sequential steps that are usually repeated several times until copulation takes place [1–3]. While performing this elaborate courtship ritual, both partners receive a plethora of information in form of chemical and physical signals processed by their olfactory, gustatory, visual, auditory, and mechanosensory organs. These cues determine the courtship behavior and, through their integration in the brain, provide to both partners information about gender, conspecificity, receptivity, and even sexual fitness [4].

The protein encoded by the *white* (*w*) gene of *Drosophila* is a transmembrane ABC transporter involved in the uptake of guanine and tryptophan, which are indispensable precursors in the synthesis of red (drosopterins) and brown (the ommochrome xanthommatin) *Drosophila* pigments [5–7] (Figure 1). The absence of pigments in the eye of flies without a functional *w* gene results in ommatidia without optical insulation. Hence the vision of such white-eyed flies is impaired, especially at high light intensities. These flies have a significantly reduced visual acuity [8] and show no response in optomotor tests [9]. Their photoreceptors receive about 19 times more light than those of wild-type flies [8], and their electroretinograms are abnormal [10]. Since guanine is further required for the synthesis of dopamine and serotonin [11,12] and tryptophan is also a precursor of serotonin [13] (Figure 1), *w* mutants display altered levels and distributions of these neurotransmitters [14]. Accordingly, mutations affecting the *w* function have an impact on the neural control of various behaviors, independent of proper eyesight [15–19].

Specifically, *Drosophila* males that ectopically overexpress the *w* gene were shown to vigorously court each other and form courtship chains, a behavior called chaining [20,21], which implied a function of *w* in the control of courtship behavior. Subsequent work suggested that increased male-male courtship results from overexpression-induced mislocalization of White protein within the cells that normally express it [22]. In apparent agreement with this hypothesis, *w* mutant males did not show enhanced male-male courtship and consequently did not chain [20–22].

Yet contrary to this hypothesis, we demonstrate here that the absence, or insufficient levels, of extra-retinal White protein causes enhanced male-male courtship, observed both in chaining and single-choice courtship assays. We further show that not only visual acuity of *w* mutant males is impaired, but that their behavior in daylight largely results from an overflow of light, which impedes the proper interpretation of earlier results on courtship behavior of *w* males [20–22]. In addition, we demonstrate that ‘rescue’ of *w* mutants by the standard *mini-white* gene does not rescue all behavioral aspects of wild-type behavior. Finally, we present results which suggest that the enhanced male-male courtship of *w* mutants is caused by a generally increased sexual arousal rather than the male’s inability to receive or process repellent sensory signals from the courting male.
Materials and Methods

Fly Stocks

The genotype of transgenic Poxn-pRes flies is ΔXB:6; PoxnΔM22-B5/PoxnΔM22-B5ΔPB:96.2 [4,23]. In these PoxnΔM22-B5 flies that are deficient for the Poxn gene, all Poxn functions are rescued by two Poxn transgenes, ΔXB:6 and ΔPB:96.2, except those required for taste bristle development [4]. It should be noted that both ΔXB:6 and ΔPB:96.2 [23] include a mini-white marker gene in the pW6 vector [24]. The genotype of mini-white flies is P(mini-white UAS-Poxn-6) inserted on the second chromosome [25,26]. Since two independent mini-white insertion lines produced comparable results, we present results from only one line. The mini-white mutant was kindly provided by William Pak. The w1118 mutant and a translocation of the white locus to the Y chromosome, Dp(1;Y)w[P{mini-white}]yBL7606, were obtained from the Bloomington stock center.

Courtship Assays

All courtship assays, manipulations of object flies, and fly husbandry were performed as described [4]. The courtship vigor index, cvi, is defined as fraction of time the male spent courting from courtship initiation, when first extending and vibrating a wing (love song), until copulation or the end of observation at 10 minutes [4]. Any of the following behaviors were scored as courting: wing vibration, tapping, licking, bending the abdomen, orienting, following with extended wings, and scanning. In daylight experiments with blind mini-white or w1118 males, bilateral wing vibration as well as wing extension that was not directed towards the female was scored as courting since these males never displayed such behaviors in the absence of a female in the courtship chamber. Periods of courtship were measured by eye displayed such behaviors in the absence of a female in the courtship chamber. Periods of courtship were measured by eye.

is justified, as all courtship experiments presented here and in [4] were performed at the same time.

Statistical Methods

Statistics was performed by the use of GraphPad Prism statistics software, applying the nonparametric Kruskal-Wallis test, which does not assume a Gaussian distribution of the results, followed by Dunn's post test to compare all pairs of groups. Instead of indicating significance markers in figures, we provided p values in the text and used double standard error bars in figures to facilitate “comparison by eye” [27].

Results

Reduced Sexual Arousal of w1118 Males in Daylight

In daylight, the sexual arousal, induced by the presence of a receptive virgin female, was dramatically reduced in w1118 males compared to Ore-R males, as measured by their courtship vigor index cvi (Figure 2A, p<0.001; for cvi, see Material and Methods). This observation is consistent with the inability of w males to follow decamping females in daylight [4]. Accordingly, the low cvi of w1118 males in daylight was raised again to that of wild-type males by a mini-white gene expressed in the eye or by a genomic w gene translocated to the Y-chromosome (P(mini-white) or Dp(1;1)w[yBL7606] in Figure 2A). In the dark, however, w1118 males displayed wild-type behavior, as indicated by a cvi comparable to that of Ore-R males (Figure 2A, p=0.14). This is explained by the fact that males, in search of a decamping female, switch in the dark to a scanning strategy [4], independent of their w gene activity. Similarly, the cvi of w1118 males, rescued by a mini-white or genomic w gene, did not differ significantly in the dark from the cvi of Ore-R males (Figure 2A, p=0.11 or 0.51).

Astonishingly, the cvi of w1118 males towards intact females in daylight was also considerably lower than that of blind mini-white males (Figure 2A, p<0.001), whose blindness is caused by a block in the synthesis of the retinal chromophore of rhodopsin [28,29]. Moreover, unlike wild-type Ore-R and mini-white males that courted females, which had been decapitated to prevent them from decamping [4], very intensely in daylight, w1118 males courted such females in daylight three times less vigorously (Figure 2B, p<0.001). Removal of the retinal chromophore by combining the
**Figure 2.** \(w^{1118}\) flies are not only optomotor blind but also dazzled by light. Courtship vigor indices were measured in single-choice courtship assays with mature males of indicated genotypes and receptive (A) or decapitated (B) Ore-R virgin females in daylight (yellow columns), under dim red light (red columns), or under low-intensity light conditions (orange columns). The numbers below the columns indicate the number of couples observed that initiated courtship, and error bars always represent double standard errors of the mean. Data for \(\text{ninaB}^{360d}\) males in panel A are from [4].

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\(\text{ninaB}^{360d}\) with the \(w^{1118}\) mutation enabled \(w^{1118}\) males again to court both decapitated and intact females in daylight with a cvi comparable to that of \(\text{ninaB}^{360d}\) males (cf. \(w^{1118}, \text{ninaB}^{360d}\) with \(\text{ninaB}^{360d}\) males in Figure 2A, \(p = 0.61\); and in Figure 2B, \(p = 0.49\)). In the dark, however, ‘blind’ single- or double-mutant males (\(w^{1118}, \text{ninaB}^{360d}\), or \(w^{1118}, \text{ninaB}^{360d}\)) showed the same behavior as Ore-R males in both assays, i.e., with intact (Figure 2A) or decapitated females (Figure 2B). Finally, \(w^{1118}\) males whose eye-pigmentation, and hence photoreceptor insulation, was rescued by.

**Figure 3.** Lack of extra-retinal White function influences sexual orientation of males. Courtship vigor indices were measured in single-choice courtship assays with mature males of indicated genotypes and decapitated males (hatched columns) or decapitated females (filled columns) in the dark (red columns) or in daylight (yellow columns). The numbers below the columns indicate the number of males observed that initiated courtship. Data for Ore-R males are from [4].

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means of mini-white or genomic w+, courted decapitated females very vigorously, independent of light conditions (Figure 2B).

These observations led us to hypothesize that in daylight w^{1118} males are not only lacking visual acuity and hence are optomotor blind, but that, in contrast to ninaB^{30d} males, they are also dazzled by an excess of light because of the absence of photoreceptor insulation. To test this hypothesis, we evaluated the performance of w^{1118} males towards females under low-light intensities, at illuminances of 11 lx and 21 lx, which are about 40 and 20 times lower than standard daylight laboratory conditions. Since these illuminances do not represent darkness for the flies, wild-type males, supplemented with mini-white gene, showed a clear preference toward decapitated females (Figure 3, p<0.001 in both cases). However, while w^{1118} males, rescued with a genomic w+ gene, displayed the same discrimination against decapitated males as Ore-R males (Figure 3, p=0.46), w^{1118} males, supplemented with mini-white, showed a significantly increased cvi towards decapitated males as compared to Ore-R males (Figure 3, p<0.01). Thus, w^{1118} flies, rescued by a mini-white rather than a genomic w+ gene, displayed a clear reduction of sexual discrimination in the dark as well in daylight.

Reduced Sexual Discrimination of w^{1118} Males

We next examined the sexual orientation w^{1118} males. As in daylight the overflow of light reduces the sexual arousal of w^{1118} males, they were tested for their courtship preference in the dark and, to avoid the influence of repellent feedback signals from the courted male, with decapitated object males and females in single-choice courtship assays [4]. The cvi of w^{1118} males towards decapitated males was enhanced as compared to that of Ore-R males (Figure 3, p<0.001) and equaled the cvi of w^{1118} males towards decapitated females (Figure 3, p=0.49). Strikingly, while supplementation of w^{1118} males with a genomic w+ gene reduced their elevated arousal towards decapitated males to the control level of Ore-R males (p=0.22), their endowment with a mini-white gene clearly did not (Figure 3, p<0.01). As a control, males carrying the mini-white gene in a w+ background behaved like Ore-R males (Figure 3). In addition, neither the inactivation of w nor its rescue by a mini-white or genomic w+ gene affected the high cvi of males towards decapitated females, which was equal to that of Ore-R males (red filled columns in Figure 3, p=0.31 for w^{1118} vs. Ore-R, p=0.10 for w^{1118}, P{mini-white} vs. Ore-R, and p=0.27 for w^{1118}, Dp(1;Y)w^{y+} vs. Ore-R).

In daylight, w^{1118} males, rescued by a genomic w+ or mini-white gene, showed a clear preference toward decapitated females (Figure 3, p<0.001 in both cases). However, while w^{1118} males, rescued with a genomic w+ gene, displayed the same discrimination against decapitated males as Ore-R males (Figure 3, p=0.46), w^{1118} males, supplemented with mini-white, showed a significantly increased cvi towards decapitated males as compared to Ore-R males (Figure 3, p<0.01). Thus, w^{1118} flies, rescued by a mini-white rather than a genomic w+ gene, displayed a clear reduction of sexual discrimination in the dark as well in daylight.

Lack of Extra-retinal White Protein in Males Induces Chaining

Since an effect of White overexpression on courtship behavior has previously been demonstrated for groups of flies [20,21], we further examined w^{1118} males in chaining assays, in which eight sexually naive males were observed in daylight in a 10 mm x 35 mm Petri dish filled with a 6 mm agar layer [4]. Under these experimental conditions, w^{1118} males, like wild-type Ore-R males, did not court each other, and thus no courtship chains were observed (Figure 4A), in agreement with previous observations [20–22]. However, it is important to emphasize that w^{1118} males are not expected to display a chaining behavior because (i) they are optomotor blind and hence are unable to follow other flies using visual cues [4], and (ii) excess of light under standard daylight conditions decreases their sexual arousal (Figure 2). In contrast, w^{1118} males rescued by a mini-white gene (w^{1118}, P{mini-white}) started courting each other only a few minutes after they had been placed into the Petri dish, whereas w^{1118} males rescued by a genomic w+ gene (w^{1118}, Dp(1;Y)w^{y+}) did not chain (Figure 4A). The chaining indices of groups of w^{1118}, P{mini-white} males, defined as the percentage of time three or more males form a chain during a 10-minute observation period [30], were not very high, and the chains consisted usually of only 3 or 4 males. This behavioral phenotype of w^{1118} males carrying a mini-white gene was further rescued by a copy of the genomic w+ gene or by replacing the w^{1118} chromosome with a w+ chromosome from Ore-R flies (Figure 4A). Thus, the missing or reduced discrimination between decapitated females and males in the dark (Figure 3) is similar to
consistent with the observation that Poxn-pRes males display equally high cvi towards decapitated males and females in the dark [4], like w^{1118}, males rescued by a mini-white gene (Figure 3). Yet, contrary to expectation and this hypothesis, double mutant w^{1118}, Poxn-pRes males, which include two copies of a mini-white gene (Materials and Methods), displayed a considerably enhanced chaining behavior (Figure 4A,B). Moreover, all w^{1118}, Poxn-pRes males participating in forming courtship chains almost all the time (Movie S2). We conclude that the reduced sexual discrimination in taste-deficient Poxn-pRes males is strongly enhanced by, and hence independent of, the lack of extra-retinal White protein in w^{1118}, Poxn-pRes males.

Increased Sexual Arousal of w Males Towards both Females and Males

Unlike Ore-R males, whose cvi shows a clear preference for decapitated females as compared to decapitated males [4], no such preference is observed with taste-deficient Poxn-pRes males or w^{1118} males in the dark (Figure 5A). However, this lack of discrimination between decapitated females and males in the dark may have different reasons for taste-deficient Poxn-pRes males and w^{1118} males deprived of vision. For Poxn-pRes males are strongly inhibited from courtship initiation, as evident from the reduced fraction of males initiating courtship during the observation period (Poxn-pRes vs. Ore-R in Figure 5B, p<0.05 in both cases) and from a considerably extended time (latency) till courtship initiation towards females but not males (Figure 5C, p<0.01 and p = 0.07). By contrast, all w^{1118} males initiate courtship towards both decapitated females and decapitated males (Figure 5B), while their latency till courtship initiation is slightly increased (p = 0.04) towards decapitated females, but unchanged (p = 0.82) towards decapitated males (w^{1118} vs. Ore-R in Figure 5C). Thus, these two genetic manipulations, which both resulted in the loss of sexual preference, may have opposite effects on the state of sexual arousal in the courting male. While for a courting male the lack of gustatory perception reduced the attractiveness of a female and to some extent of a male [4], the absence of w functions increased its sexual excitability by a decapitated male in the dark. In daylight, on the other hand, lack of extra-retinal White also enhanced the sexual arousal of the courting male towards both decapitated males (Figure 3) and intact males, as reflected by the drastically augmented chaining of w^{1118}, P[mini-white] and w^{1118}, Poxn-pRes males (Figure 4 and Movie S2).

To test if lack of extra-retinal White increases the sexual arousal of males in general, i.e., also towards females, we took advantage of Poxn-pRes; Or83b^{2} males which can neither smell nor taste [4]. In daylight, these males display a rather low cvi towards receptive females in single-choice courtship assays (Figure 5D) [4]. Yet, they court decapitated females very vigorously (Figure 5D). These observations indicate that vision alone does not provide sufficient sexual stimulation to maintain high levels of sexual arousal after the intact female decamps [4]. In agreement with a sexual arousal of w^{1118} males supplemented by mini-white, the lack of extra-retinal White protein increased the courtship vigor of w^{1118}, Poxn-pRes; Or83b^{2} males two- to three-fold towards intact receptive females in daylight (Figure 5D, p<0.001). The cvi towards decapitated females of these males, as compared to Poxn-pRes; Or83b^{2} males, could not be significantly enhanced as the cvi of the latter was too close to 1 (Figure 5D).

the difference in chaining behavior shown in Figure 4A. In both cases, this deficiency is evident in w^{1118} males only when they are supplemented with a mini-white gene but not when they are endowed with a genomic w^{+} gene. Since large parts of the control regions of the genomic w^{+} gene have been deleted in the mini-white gene [26], this difference in behavior is interpreted in terms of a reduced or absent extra-retinal expression of White protein in w^{1118} males supplemented with a mini-white gene.

As the chaining phenotype of w^{1118} males rescued by a mini-white gene resembled that of taste-deficient Poxn-pRes males (Figure 4A) [4], we assumed that the w^{1118} mutation may affect the processing of sex-specific gustatory information. This hypothesis was further
Discussing

Influence of Light Over-flow on Courtship Behavior of White Males

We have shown in this study that in daylight, w1118 males court receptive virgin females with a cvi much lower than that of Ore-R males, as expected from the absence of proper vision on which a male depends to follow a decamping female (Figure 2A). However, in daylight these males also showed a low sexual arousal when facing decapitated females unable to decamp (Figure 2B). As expected, this striking behavioral phenotype of w null mutant males could be reverted to wild-type by rescue of the insulating eye-pigments with a mini-white or genomic w+ gene. Surprisingly, however, the cvi of these w1118 males was also (i) much lower than that of truly blind ninaB0680d males, whose phototransduction is completely inhibited, and (ii) could be elevated to that of ninaB0680d males by a drastic reduction of the light intensity during the test (Figure 2). As the NinaB protein is involved in the synthesis of retinoids, one might argue that the use of ninaB mutants has effects other than those caused by the block in chromophore synthesis of rhodopsin in photoreceptors. Such additional functions would also be impaired in ninaB0680d mutants and might act upstream of extraretinal White protein. Yet, it appears that such functions do not influence male courtship as no difference in courtship behavior of ninaB0680d and Ore-R males, whose eyes were covered with black paint, were observed either in daylight or in the dark [4]. In the dark (dim red light), w1118 males behaved like Ore-R males (Figure 2). Therefore, we concluded that in daylight w males are dazzled by the over-flow of light and that over-activation of the photoreceptors suppresses their sexual arousal. In this context, it is remarkable that the sensitivity to the anesthetic halothane of w1118 flies is increased in ambient light as compared to darkness, which suggests that light over-excitation affects the general state of arousal of w flies in daylight [31].

Lack of Extra-retinal White in Males Increases their Sexual Arousal

In the absence of light over-excitation in the dark and of the repressive male feedback behavior prevented by decapitation, the preferred sexual orientation of males for females was completely abolished in w1118 males (Figure 3). Wild-type sexual orientation was restored in these males by rescue with a genomic w+ gene (w1118; Dp(1;3)Ya3y) but not with a mini-white gene (Figure 3). In daylight, however, w1118 males supplemented with a mini-white gene showed a clear preference for decapitated females, a behavior that is explained by the sexual discriminatory function of the restored vision in the courting male [4], but male-male courtship was elevated (Figure 3). Interestingly, w+; Dp(1;3)Ya3y+ males displayed a slightly but significantly increased male-male courtship in daylight in this assay (data not shown). This increase in cvi probably results from the presence of two genomic w+ copies in these males and the concomitant overexpression of White protein, in line with previous observations [20–22]. In the more complex chaining assay, w1118 males supplemented with a mini-white gene engaged in male-male courtship and chaining (Figure 4). Note that in addition to the results presented in Figure 4, we tested six other mini-white lines in a w1118 background and observed chaining behavior in all experiments (data not shown). Therefore, we argue that the mini-white gene used here and in numerous other studies, which is fully expressed in the eye, lacks enhancers of the wild-type w gene that express it in the CNS and/or PNS at levels sufficient to rescue all w functions and mutant behaviors. Thus, this extra-retinal w function is crucial to rescue all mutant behaviors. It follows that an extra-retinal function of White is important to control the sexual arousal in males. In addition and more generally, our findings caution against interpretations of results obtained in behavioral studies of w mutant flies rescued only by a mini-white gene. As the lack of extra-retinal White increases the sexual arousal of males also towards females, the observed male-male courtship and chaining behavior of w1118 males, supplemented with mini-white, may be a secondary effect of an increased overall sexual excitability of these males. In addition, we would like to emphasize that the excessive and relentless chaining behavior of males [Movie S2] is observed only when in addition to the reduction or removal of extra-retinal White in w1118; P(mini-white) males, which stimulates their sexual arousal (Figure 5), gustatory reception, which inhibits male-male courtship [4], is abolished in w1118; Poxn-pRes males by the substitution of wild-type Poxn with the appropriate Poxn transgenes (Figure 4).
Since White protein is important for the cellular import of tryptophan, a precursor of serotonin [13], and serotonin levels are affected in w mutant flies [14], it is tempting to draw parallels between our observations and those made in rats, cats, and rabbits where the reduction of serotonin levels increased the sexual arousal of males [32-34]. These tried to mount not only females but also conspecific males. We suggest, therefore, that the w mutant phenotypes observed in our assays may at least in part be caused by lower levels of serotonin. In line with this suggestion, recent work showed that w mutant flies display an elevated phototactic personality and individual reaction to light stimuli, while this enhanced reaction is suppressed by w-dependent serotonin [35]. Therefore, it would be interesting to know whether and which behaviors other than courtship are affected by the lack of extra-retinal White and if these could also be induced by pharmacological reduction of serotonin levels.

It has been proposed that increased dopamine levels in Drosophila lead to an increase in general arousal, which results in an elevated behavioral responsiveness [36] and, more specifically, in an enhanced courtship vigor [37] as well as male-male courtship [38]. Surprisingly, however, a similar effect is achieved by a reduction of dopamine levels, which appears to decrease the threshold of arousal [36], thus rendering flies responsive to a lower intensity of stimuli. Although the effects of lower dopamine levels on courtship behavior still need to be confirmed, results with w1118 males, whose serotonin as well as dopamine levels are reduced [14] (Figure 1), are consistent with such a bimodal function of dopamine.

Models

The conspicuous mutant phenotype of the w gene was discovered by Morgan in 1910 and was the first reported Drosophila mutant [39]. Ever since, the w gene has been extensively used as a convenient genetic marker. Despite this fact, characterization of the w gene at the molecular level, other than determining its DNA sequence and, derived from it, that of its protein, has been hampered, mainly because the protein is expressed at very low levels. Thus, expression outside the eye, where its expression is obvious from its mutant phenotype, is not well documented. Hence, it is unclear where w is expressed in the central nervous system (CNS) and peripheral nervous system (PNS) of the adult fly. According to FlyBase, w is expressed at low to extremely low levels in the brain and CNS but no evidence for its expression in the PNS is known.

Therefore, based on the behavioral phenotypes of w mutants reported here, we suggest that the lack of White protein in the CNS, other than eye and ocelli, and perhaps in the PNS, increases the overall sexual arousal of males, leading them to indiscriminately court decapitated males and females in the dark, or to chain in daylight when placed in groups of males. This increased sexual arousal of w mutant males may result from an elevated alertness (Figure 6A), a reduction of the threshold for stimuli that elicit courtship (Figure 6B), or an enhanced sensitivity to pheromone stimuli (Figure 6C). While the first two models imply an augmented sensitivity for courtship in the CNS of courting w males, the third model suggests that these males exhibit an accelerated processing by the CNS of courtship-relevant information and perhaps an increased activity of PNS neurons that receive sensory stimuli for courtship initiation and maintenance. In each model, however, the interval of latency till sustained courtship is reduced from t in wild-type to t in w1118 mutant flies (Figure 6). Although in reality a combination of these models may be relevant, further studies are required to discriminate between them. It should be emphasized that the effect of reduced or absent extra-retinal White protein on a male’s state of sexual arousal could be indirect. Thus, the absence or reduction of the extra-retinal white function might affect a network of the nervous system that is not involved in courtship control but whose state influences the circuitry regulating courtship behavior.

Supporting Information

Movie S1 Courtship assay clip of a wild-type Ore-R male after he initiated courtship. Experiments were performed under illumination of 21 lx (first part) and 11 lx (second part). Under both conditions, the male orient towards and follows the female and does not switch to scanning behavior after the female decamps. The second part of the clip was recorded by the use of the “low-illumination mode” of the camera.

Movie S2 A chaining assay video showing eight sexually naïve w1118; Poxn-pRes males in a small Petri dish. All males participate in forming a courtship chain. The video was recorded 3–4 minutes after the males have been placed together.

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Author Contributions

Conceived and designed the experiments: DK. Performed the experiments: DK. Analyzed the data: DK MN. Contributed reagents/materials/analysis tools: WB. Wrote the paper: DK MN.

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