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Gametic incompatibilities between races of *Drosophila melanogaster*

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Reproductive-isolating mechanisms between nascent species may involve sperm–egg recognition and have been best described in externally fertilizing organisms where such recognition is essential in preventing undesirable fertilizations. However, reproductive barriers in internally fertilizing species differ in significant ways, and a direct role for sperm–egg interactions has yet to be demonstrated. Females of many strains of *Drosophila melanogaster* from Zimbabwe, Africa, do not mate readily with cosmopolitan males. This polymorphism in mate choice is postulated to represent incipient speciation. We now report that, in one direction, crosses between the above populations produce far fewer offspring than reciprocal crosses due to a lower rate of egg hatch. We established that egg inviability in these crosses was due to defects in fertilization. Thus, even in taxa with internal fertilization, gametic incompatibility may be a mechanism relevant to reproductive isolation during incipient speciation.

Keywords: reproductive isolation; gametic incompatibility; *Drosophila melanogaster*; incipient speciation

1. INTRODUCTION

The isolating mechanisms driving speciation have concerned evolutionary biologists for a long time. Studies of post-mating isolation have made strides in identifying the genetic basis of speciation in sibling and closely related species. To date, these studies have largely focused on interspecific hybrid sterility and inviability (Hutter & Ashburner 1987; Wu & Palopoli 1994; Davis *et al*. 1996; True *et al*. 1996; Coyne & Orr 1998; Coyne *et al*. 1998; Hollocher 1998; Ting *et al*. 1998; Sawamura *et al*. 2000). Similarly, studies within populations at the nascent or early stages of speciation could provide important clues about the mechanisms affecting isolation. *Drosophila melanogaster* is currently known to have two behavioural races, i.e. the cosmopolitan (M for *melanogaster*) populations and the African (Z for Zimbabwe) populations (Wu *et al*. 1995). The M racial type has a worldwide distribution, while the Z populations have so far only been found in sub-Saharan Africa (Wu *et al*. 1995; Hollocher *et al*. 1997). These two races exhibit significant behavioural isolation (Wu *et al*. 1995; Hollocher *et al*. 1997). When given the choice, females from most Z populations do not mate with males from M populations, whereas reciprocal crosses result in little or no behavioural isolation. The specific behaviours that allow Z females to discriminate between Z and M males are currently unknown. Because the focus of these studies was the behavioural aspects of the Z/M system, they were not designed to detect forms of post-mating isolation. However, it was noted that hybrid crosses in both directions were viable and fertile. We quantified the viability of eggs in inter-racial crosses and can now report that, concurrent with the behavioural isolation, the sperm of M males are not fully compatible with the eggs (and/or the reproductive tract) of Z females.

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2. MATERIAL AND METHODS

(a) *Stocks*

(i) *Iso-female lines*

Two races of *D. melanogaster* were used in this study; these were the cosmopolitan (M lines) and Zimbabwe (Z lines) races. Five standard M lines (Fr, Hg, OR, GF(Id)-5E and Tai) and two standard Z lines (Z30 and Z53), in which females deferentially mate with males from their own populations, were used. Fr was collected in France, Hg was collected in California, both in the 1980s, OR was collected in Oregon in the 1920s, GF(Id)-5E was collected in Indiana in 1997 and Tai was collected from the Ivory Coast in the 1970s. The five M-type lines displayed the characteristic M-type behaviour in which females show no mate choice. The Z lines were collected in Zimbabwe and the surrounding countries Botswana, Zambia and Malawi in 1990 (Begun & Aquadro 1993). The behaviours of the Fr, Hg, Z53 and Z30 iso-female lines are described in Wu *et al*. (1995). The behaviour of all lines was tested several times during the course of these experiments and all lines were shown to possess behaviour consistent with that described in Wu *et al*. (1995).

(ii) *Composite lines*

In assays measuring any facet of fitness, results from lines such as the composite lines possess the characteristic mating phenotype while minimizing the effects of inbreeding depression that often plague iso-female line use. Three of the composite lines used in this study were established from several iso-female lines. The SZ line (strong Z line) was generated from crosses between pairwise reciprocal crosses of six behaviourally strong iso-female lines, i.e. Z30, Z53, Z56, Z(S)2, Z(H)32 and Z(S)11 (see Hollocher *et al*. 1997). Similarly, the IZ line (intermediate Z line) was generated from crosses between pairwise reciprocal crosses of behaviourally intermediate iso-female lines, i.e. Z(S)6, Z(S)28, Z(S)29, Z(S)40, Z(H)33, Z(H)42, LA 69 and OK59. The IZ line represents a subset of Z lines, the females of which display an intermediate preference for Z-type males compared to the Z lines (Hollocher *et al*. 1997). Six cosmopolitan iso-female lines (Fr, Hg, Can S, OR, Tiw (from Taiwan) and

Aus (from Australia)) were used to generate the M line in the same manner as the IZ and SZ lines. With the exception of the GF(Id)-5E and Tai lines, all iso-female lines present in the study were represented in the composite lines. Prior to their use, the lines were maintained for one year at moderate population sizes (> 500) under standard laboratory conditions of a two-week generation cycle at 23 °C under a 12 L:12 D cycle on standard corn meal fly culture media.

(b) *Egg viability assay*

Four- to six-day-old virgin flies of each line or cross were allowed to mate and feed on an excess of yeast added to agar/molasses plates for *ca.* 36 h. After this 36-h pre-collection period, flies were transferred to fresh agar/molasses yeast plates and eggs were collected 2 h later. One hundred young embryos from the 2-h embryo collections were removed from the plates each day for three days via a small, fine paintbrush and placed on a fresh agar/molasses plate. These plates were checked at 24 and 72 h and, at these times, the numbers of eggs that had not hatched were recorded. Eggs that had not hatched by 72 h were scored as inviable. In total, 300 embryos were scored for hatching per racial line or inter-racial cross. The data were analysed using Tukey *post hoc* analysis and an SAS statistical analysis program.

(c) *Fertilization assay*

Concurrent with the measurement of egg viability, eggs from the same collection plates were fixed and stained with the monoclonal antibody DROP 1.1 (Karr 1991; Graner *et al.* 1994). Fertilization was scored by the presence of a coiled sperm tail in the anterior portion of an egg. Partially fertilized eggs were scored by the presence of any portion of the sperm seen to be outside of the egg, indicating incomplete sperm penetration. A total of 300 embryos per racial line and inter-racial cross were scored. The data were analysed using Tukey *post hoc* analysis and an SAS statistical analysis program.

(d) *Sperm viability assay*

Viability was evaluated using both visual examinations of sperm motility and a live/dead sperm viability assay (König *et al.* 1995). This fluorescence-based assay (Molecular Probes, Inc., Eugene, OR, USA) contained propidium iodide, a conventional stain that indicates dead or dying cells and Syber 14® dye, a membrane-permanent nucleic acid stain. Cells that were Syber 14® positive but propidium iodide negative indicated viable cells. Together, they enabled us to distinguish living cells from dead cells. Virgin flies were aged for four to five days before being placed in bottles for mass mating. Three to five days after mating (when females were seven to ten days old), 30–40 females from each cross were dissected and their spermathecae and seminal receptacles were removed and stained. The sperm were then removed and viewed under both fluorscein isothiocyanate (FITC) (Syber 14®) and rhodamine (PPI) filters. Images were obtained using a CCD camera and IPLab, an image processing application. The images were displayed and printed using Adobe PhotoShop 5.0.

3. RESULTS

We examined two parameters of pre-larval development, i.e. percentage egg hatch and the percentage of successful fertilizations (see § 2). Together, these parameters provide information on post-mating isolation between these two racial populations. We report on the values for both iso-female (Z and M lines) and composite lines (the SZ, IZ and M lines) for all experiments (table 1) (also see § 2).

Approximately 90% of the eggs laid in both Z- and M-type intra-racial crosses were found to be viable (table 1). Similarly, egg viability in crosses between M females and Z males (M×Z crosses) was *ca.* 84% (table 1). However, on average we observed a 40% reduction in egg viability in crosses between Z females and M males (Z×M crosses) (table 1). Overall, the differences between the Z×M and all other reciprocal crosses were significant (Tukey *post hoc* analysis, $p < 0.001$) (table 1), although there was large variation observed in this trait in the Z×M crosses (range 39–85% egg hatch) (table 1).

The non-reciprocal nature of egg viability led us to examine fertilization in these crosses closely. We employed a sperm tail-specific monoclonal antibody in order to evaluate fertilization (Karr 1991; Graner *et al.* 1994). A successful fertilization is one in which the entire sperm (*ca.* 1.75 mm in *D. melanogaster*) (Pitnick *et al.* 1995) enters the egg and folds in a stereotypical manner (Karr 1991, 1996; Karr & Pitnick 1996). We considered either the absence or partial entrance of a sperm into an egg as an 'unsuccessful' fertilization (table 1). Using this protocol, we ascertained that over 95% of the eggs from intra-racial matings of either the Z or M populations were fertilized (table 1). Similar values for fertilization were found in crosses between M females and Z males (table 1). In contrast, the average number of successful fertilizations was significantly reduced in crosses between Z females and M males (Z×M crosses) (table 1) relative to their parental lines and reciprocal crosses (Tukey *post hoc* analysis, $p < 0.001$). In addition, there was a large increase in the number of partially fertilized eggs (see § 2) (figure 1) in the Z×M crosses (2–13%) relative to the percentage observed in the M×Z crosses (*ca.* 1%) (table 1). It appears that sperm may reach the egg but have difficulty penetrating. It is possible the M-type sperm are so damaged during the storage process that they are unable to enter the egg completely. Figure 1 shows one such instance in which the sperm failed to penetrate the egg completely. Such eggs never develop. Variation in both egg hatch and fertilization was clearly greatest in the Z×M crosses. For example, reciprocal crosses between the Z30 and Fr iso-female lines (table 1) were not different (90.45 versus 93.75%), yet all other crosses were consistently different. The mating behaviour of the Z30 line was rechecked and still found to discriminate strongly against M-type populations, eliminating the possibility of contamination in this line. Thus, a reduction in egg viability correlates quantitatively with fertilization ability. We shall address the variability among Z×M crosses below.

In *Drosophila*, as in many insects, sperm must be stored prior to fertilization and, therefore, the processes involved in sperm storage and release could affect sperm function. In order to address these possibilities, the motility of sperm released from the females' two main storage organs, i.e. the ventral receptacle and the spermatheca, was monitored upon dissection by visual inspection and fluorescence microscopy. We determined that sperm displayed typical sinusoidal movements and beat frequencies in all reciprocal

Table 1. *Egg viability and fertilization assays*

(Both egg viability measured as percentage egg hatch and the percentage of fertilized eggs gave similar results. The differences between the inter-racial crosses were highly significant except in the case of FrV3-1 and Z30. Iso-female line variation may indicate that, even within a population, the ability of males to fertilize eggs may vary. The names and values marked with an asterisk represent data from the composite lines and crosses between the composite lines. A large percentage of the eggs laid in Z×M crosses were found to be partially fertilized, thereby implicating sperm–egg interactions. A total of 300 eggs were surveyed for each cross and line. Z×M crosses versus M lines, M×Z crosses, F1(MZ), F1(ZM) and Z lines were significantly different for the percentage egg hatch (Tukey *post hoc* analysis, $p < 0.001$ for all comparisons with differences between all other crosses non-significant). Similarly, comparisons of the percentage of fertilized eggs in Z×M crosses versus M lines, M×Z crosses, F1(MZ), F1(ZM) and Z lines were significantly different (Tukey *post hoc* analysis, $p < 0.001$ for all comparisons with differences between all other crosses non-significant). Z×M crosses versus M lines, M×Z crosses, F1(ZM) and Z lines were significantly different for the percentage of partially fertilized eggs at the $p < 0.01$ level, whereas Z×M crosses versus F1(MZ) was only significant at the $p < 0.05$ level (Tukey *post hoc* analysis) (differences between all other crosses non-significant) *n*-values are given in parentheses for the percentage of total eggs partially fertilized.)

cross	percentage of fertilized eggs (±s.e.)	percentage hatch (±s.e.)	percentage of total eggs partially fertilized
M lines			
M line*	98.7 ± 0.3*	93.7 ± 2.7*	0.0*
Hg(Ca)	95.4 ± 1.3	95.7 ± 1.8	0.0
OR	93.7 ± 1.5	89.3 ± 2.0	1.7 (5)
FrV3-1	97.6 ± 0.5	92.3 ± 0.9	0.0
FrV3-1×Hg(Ca)	95.2 ± 1.8	91.0 ± 0.0	0.0
Hg(Ca)×FrV3-1	96.1 ± 1.0	95.7 ± 0.3	0.0
Tai line	89.3 ± 0.2	86.7 ± 0.7	0.7 (2)
mean of means	95.14	92.22	—
M×Z crosses			
M line×SZ line*	98.2 ± 0.6*	79.0 ± 0.9*	0.0*
FrV3-1×Z30	90.5 ± 1.8	82.7 ± 0.9	0.9
Hg(Ca)×Z53	93.1 ± 3.0	78.3 ± 0.3	0.0
GF(Id)-5E×Z56	83.5 ± 0.6	94.3 ± 2.9	1.8
mean of means	91.32	83.58	—
Z×M crosses			
SZ line×M line*	49.8 ± 2.4*	39.3 ± 6.9*	9.0 (27)*
Z30×FrV3-1	93.8 ± 2.9	85.3 ± 4.9	2.3 (7)
Z53×Hg(Ca)	82.4 ± 7.4	52.3 ± 2.7	11.3 (34)
Z56×GF(Id)-5E	46.2 ± 11.6	42.8 ± 4.3	13.3 (40)
mean of means	68.03	54.95	—
F1(MZ) crosses			
F1(MSZ)*	93.9 ± 1.6*	91.7 ± 6.0*	0.0*
F1(FrV3-1×Z30)	97.6 ± 0.5	68.3 ± 3.8	0.0
F1(Hg(Ca)×Z53)	97.6 ± 0.8	80.0 ± 19.1	0.0
mean of means	96.34	80.00	—
F1(ZM) crosses			
F1(SZM)*	94.5 ± 2.0*	90.0 ± 11.7*	0.0*
F1(Z30×FrV3-1)	93.9 ± 0.2	73.0 ± 9.2	0.0
F1(Z53×Hg(Ca))	99.1 ± 0.1	76.7 ± 1.5	0.0
F1(Z56×GF(Id)5-E)	96.3 ± 2.7	94.0 ± 1.0	0.0
mean of means	95.91	83.42	—
Z lines			
SZ line*	94.9 ± 0.5*	87.0 ± 1.5*	0.0*
Z30	93.9 ± 0.5	87.3 ± 2.4	0.0
Z53	95.3 ± 2.3	75.0 ± 2.7	5.3 (16)
mean of means	94.72	83.11	—
intermediate line			
IZ line*	96.4 ± 0.4*	91.7 ± 0.7*	8.0 (24)*

crosses by visual examination (data not shown, but sperm movement is indicated indirectly by the blurring of the sperm during image capture in figure 2). The viability of sperm from these dissections was monitored using an assay that distinguishes live/viable sperm cells from dead sperm cells (König *et al*. 1995). We examined sperm in storage in the reciprocal crosses between Z56 and GF(Id)-5E, Z53 and Hg as well as between the SZ line and the M line. Inseminated females from these crosses were found to contain viable and motile sperm in all cases (figure 2).

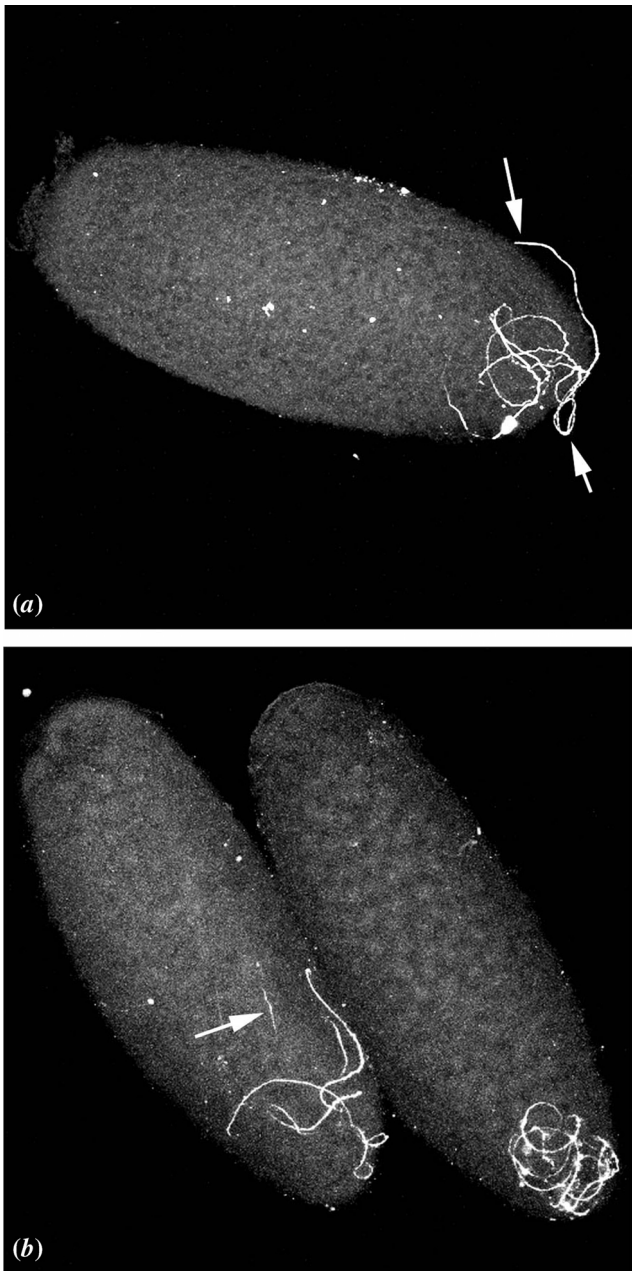

Figure 1. Three-dimensional reconstructions of fertilized eggs in two racial strains of *D. melanogaster*. (*a*) Abnormal fertilization in a Z×M cross. The arrows point to a sperm tail outside an egg, indicating a partial fertilization. The number of partial fertilizations may actually be an extremely conservative measure as eggs in which only a sperm head has penetrated the egg are also be unlikely to be seen. Therefore, some of the eggs that appear not to be fertilized may actually be those eggs in which the sperm was lost during fixation and staining. (*b*) Two fertilized eggs from a Z×M cross showing abnormal (left) and normal (right) fertilization. The arrow in the left image shows abnormal placement of a sperm tail (cf. the placement of the tail in the right image).

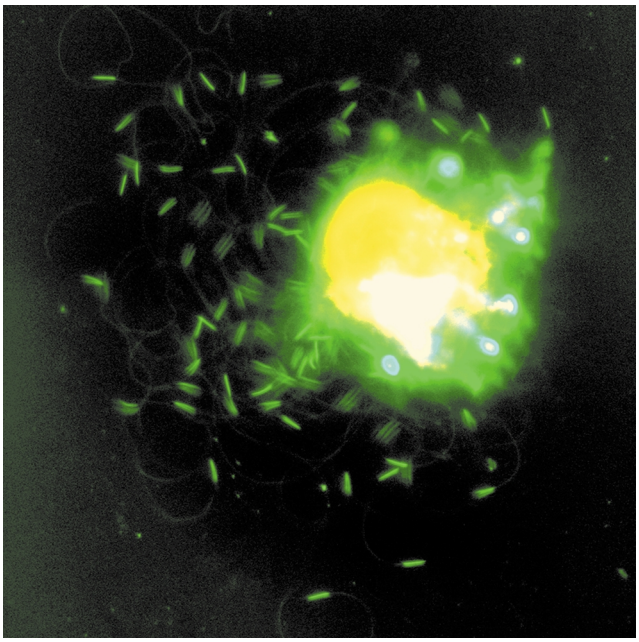

Figure 2. Sperm viability in Z×M crosses. A single spermatheca was dissected in order to release stored sperm and then stained to reveal viable sperm using the live/dead assay kit (Molecular Probes, Inc.). The small green-coloured structures indicate live sperm. No dead sperm (which would be indicated by red colour) were observed in any cross. The bright yellow colour in the centre of the figure is due to out-of-range signal from the intense green signal from the sperm tails in this region.

4. DISCUSSION

Our results suggest that, in addition to the behavioural isolation seen in the Zimbabwe lines (Wu *et al*. 1995; Hollocher *et al*. 1997), females are in effect undergoing another round of mate recognition at the cellular level. The strength of the behavioural recognition in the African populations varies, and we have shown here that there is also variation in the capacity of Z eggs to be fertilized by M-type sperm. There is evidence that the strength of the behavioural and cellular incompatibilities may be correlated as the IZ line (see § 2) displays both intermediate behavioural isolation and an intermediate level of successful fertilizations (table 1). The variation in fertilization and egg hatch observed in the laboratory indicates population-wide variation. This line-to-line variation is not unexpected at the nascent stage of racial and species differentiation when the genetics of reproductive isolation are transiently polymorphic.

We saw three different fertilization phenotypes in the Z×M crosses: complete fertilization (figure 1 and table 1), partial fertilization (figure 1 and table 1) and unfertilized eggs. There are two non-mutually exclusive explanations for these results: (i) sperm may be partially incapacitated or damaged during interactions within the female reproductive tract (e.g. sperm storage) and/or (ii) sperm begin but cannot complete penetration into the egg. Either or both could explain the large number of unfertilized eggs and the partially fertilized eggs in the Z×M crosses. However, the causes of each could arise from very different sources. Damage *en route* to or during storage could only involve interactions between sperm and the female reproductive tract, while the inability of sperm to penetrate eggs could be due to either prior damage during storage or direct gametic incompatibility. In the case of sperm viability and fertilization ability, deleterious interactions with the female tract could occur during the entrance or exit of sperm from storage. Damage during

these stages could impact on general sperm physiology negatively (at levels not observed in our motility/viability assay) or could inhibit or eliminate sperm–egg contact (subsequent sperm–egg interactions involved in complete sperm penetration of the egg). Thus, female effects on sperm behaviour and function at this level cannot be entirely ruled out. However, the female reproductive tract can only be partially involved at this level as 60% of eggs are fertilized successfully. We also noted that insemination reactions were not observed in *D. melanogaster* or other members of the *melanogaster* subgroup. Insemination reactions, which are observed in many *Drosophila* and other Diptera, occur in the female reproductive tract and result in sperm inactivation. Insemination reactions often result in the formation of a congealed sperm mass that is thought to block egg laying or reinsemination physically (Patterson 1946; Patterson & Stone 1952; Ward & Heed 1970; Asada & Kitagawa 1988; Asada & Fukumitsu 1990; Pitnick *et al*. 1995; Bertram *et al*. 1996; Polak *et al*. 1998; but see also Markow 1997). We saw no evidence of insemination reactions in our crosses, consistent with previous reports in the *melanogaster* group.

Drosophila melanogaster females are known to be affected by accessory gland proteins (Chen *et al*. 1988; Wolfner 1997). These proteins are produced and secreted by the accessory glands of males and are transferred to the female along with sperm during copulation (Chen *et al*. 1988; Wolfner 1997). Accessory gland proteins such as *Acp26Aa* have been studied extensively (DiBenedetto *et al*. 1990; Aguade *et al*. 1992; Chapman *et al*. 1995; Clark *et al*. 1995; Kubli 1996; Tsaur *et al*. 1998) and are cleaved sometime after transfer to the female (Wolfner 1997). This interaction of the female reproductive tract and male product (sperm + seminal fluid) normally causes some behavioural and physiological reactions in the female. *Acps* are known to aid in sperm storage, the stimulation of egg laying and the repression of female sexual receptivity (Chen *et al*. 1988; Kubli 1996; Wolfner 1997). It is believed that *Acp*s may have evolved in part to increase male paternity in the face of multiple mating and sperm competition. This is a logical conclusion as the effect on the female makes it either impossible or simply less likely that the female will remate until at least some of the males' sperm is used. Negative interactions between seminal fluids and the female reproductive tract may be involved in reducing M male fertility in Z×M matings. Although sperm appeared to be stored normally and remained motile, these qualitative observations cannot rule out female tract interactions. For example, *Acp*s affect sperm competition and impact negatively on the number of sperm stored and, therefore, reduce the number of sperm which are available for use in fertilizing eggs (Neubaum & Wolfner 1999; Chapman *et al*. 2000). In the same way, variation in the *Acp36DE* gene itself is probably responsible for variation in the ability to defend against sperm displacement (Clark *et al*. 1995). While these mechanisms may be contributing factors in the Z/M system, the phenomena reported here between Z-type females and M-type males are manifested at the point of sperm–egg contact. The exact relationship between the phenomena reported here and contributing female reproductive tract interactions is currently being explored.

Furthermore, there is evidence that these proteins may actually be caustic to the female (Chapman *et al*. 1995; Kubli 1996; Markow 1997; Wolfner 1997). They have been implicated in reductions in the female life span and, consequently, her lifetime fitness (Partridge & Farquhar 1981; Holland & Rice 1999). Given that there is a cost associated with exposure to accessory gland proteins, it is in the best interests of the females to mediate these effects. This leads to a conflict of interest between the females and males of a population. A delicate balance of co-evolution ensues with both males and females attempting to maximize their fitness (Rice 1992, 1996, 1997; Rice & Holland 1997; Holland & Rice 1999). However, what role these evolutionary forces play in generating the variation in fertilization in the Z/M system has not been examined.

In addition to these possibilities, our results do not exclude the possibility that direct gametic incompatibility is responsible for incomplete or partial fertilizations. Indeed, there is very strong evidence that this occurs regularly in *Drosophila* in the form of cytoplasmic incompatibility, a type of reproductive isolation caused by the endocellular symbiont *Wolbachia pipientis* (O'Neill & Karr 1990; Karr 1994). In this system, sperm from infected males are incompatible with uninfected egg cytoplasm and there is no evidence that this incompatibility involves the female reproductive tract (Snook *et al*. 2000). In an incompatible cross, sperm fertilize the egg normally but karyogamy is defective and abortive egg development ensues (Lassy & Karr 1996). In this system, crosses with both incompatible and compatible males yield normal egg laying rates and normal rates of fertilization, thereby arguing that direct gametic interactions are responsible. However, while our Z lines were infected by *Wolbachia* (data not shown), they were not a contributing factor in the Z/M system because cytoplasmic incompatbility is only expressed in crosses between infected males and uninfected females (the M lines are not infected). In our system, reduction in egg hatch was only observed in crosses between Z-type females and M-type males (i.e. an infected female crossed with an uninfected male).

Furthermore, two major, male accessory gland proteins, *Acp26Aa*, which is known to affect ovulation (Heifetz *et al*. 2000) and *Acp36DE*, which is known to affect sperm storage (Neubaum & Wolfner 1999), are processed normally by the female in incompatible crosses (Snook *et al*. 2000). This suggests that the incompatibility is at the level of the gametes. Indeed, because M sperm are viable and motile in Z females (figure 2) and the majority of Z eggs laid are successfully fertilized by M sperm (over 50%) (table 1), we believe that abnormal interactions between the Z egg and the M sperm during sperm penetration contribute significantly to reproductive isolation in this system. At the cellular, developmental and structural levels, an argument can also be made that the complete penetration of the extraordinarily long *Drosophila* sperm into the egg necessarily involves direct sperm–egg interactions during fertilization (Karr 1991, 1996; Karr & Pitnick 1996). It is reasonable to assume that evolution at this level may be a player in this form of reproductive isolation.

There is ample evidence that the reproductive tract is among the most rapidly evolving of all phenotypic traits

(Eberhard 1985, 1996; Thomas & Singh 1992; Rice & Holland 1997; Hellriegel & Ward 1998; Pitnick & Karr 1998). Rice (1996), Rice & Holland (1997) and Holland & Rice (1999) showed that, when female evolution is experimentally halted, males actually become toxic to females. Is it possible that a similar situation arises when populations are evolving allopatrically? With this in mind, it is possible that, in order to maximize their own fitnesses, the coevolution of males and females may have progressed to the point where the Z females' response to these proteins merely ameliorates the effects of the Z males' product, but is caustic to the M sperm. If such a situation exists, M-type sperm, although stored and apparently viable (figure 2), may be hampered in their attempts to penetrate and, therefore, successfully fertilize Z-type eggs.

Even if sperm–female interactions are the penultimate cause of the reproductive isolation observed in the Z/M system, the effects of such incompatibilities are at least in part manifested at the level of sperm penetration and fertilization. The exact role of sperm–egg interactions versus sperm–female interactions in the generation of this gametic incompatibility could be studied using pole cell transplantation (Mahowald *et al*. 1976). In this way, the capacity of M-type sperm for fertilizing Z eggs derived from an otherwise M female reproductive tract can be determined. Another possibility is to make use of *Acp*-null phenotypes (*ex*. Neubaum & Wolfner 1999) in identifying possible interactions in other seminal products. Variations in male reproductive traits are well documented (Anderson 1982; Houde 1987; Basolo 1998) and, while the direct contributions of sperm in gametic incompatibilities have not been previously measured, variation in the sea urchin sperm protein bindin has recently been reported (Vacquier & Lee 1993; Palumbi 1999). Females of the sea urchin genus *Echinomerta* can fertilize eggs assortatively based on differences in bindin genotype, which appears to be polymorphic within a species (Palumbi 1999). Given the partial fertilization phenotype observed in figure 1 and the large quantities of sperm protein entering the egg (Karr 1996), the fertilization failure in the Z×M crosses may be due to variation in the protein(s) involved in sperm entry and localization within the egg.

This study has implications for two different fields of research. First, this work offers up a possible complication to the study of sperm competition as it appears that, at the least, this system is in possession of the ability to implement assortative fertilization (Markow 1997). Second, the inter-racial incompatibility we have described here underscores the possible role of post-mating mechanisms involving sperm–egg interactions in racial and possibly species divergence in internally fertilizing organisms. How the variation in fertilization and egg viability first arose and was then subsequently maintained in these populations can now be studied in a genetically tractable system, which should simplify the task of identifying the proteins and genes involved in the incompatibility.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.