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Fitsum Woldesellassie April 13, 2011

A study of incipient speciation between *Drosophila albomicans* and *D. nasuta*

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Abstract

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Two fruit fly species, *Drosophila nasuta* and *D. albomicans*, are closely related incipient species. These two species have allopatric distribution and are morphologically indistinguishable. Past studies reported inconsistent results on their pre-zygotic isolation but little data on their post-zygotic isolation. Notably, sex-ratio meiotic drive was observed in the F1 male from Okinawan *D. albomicans* ♀ crossed to Indian *D. nasuta* ♂ but not from the reciprocal cross. Fertile hybrids and perpetual hybrid population can be easily made between these two species in lab, thus questioning their status as separate species. Here, I address the issue of speciation status by assessing the pre-mating and post-zygotic isolations between *D. albomicans* and *D. nasuta*. We assayed the courtship behavior by direct observation and the mating frequency by multiple choice test. The courtship behavior between these two species did not differ from that within species, and the mating frequency was also similar between and within species. Overall, there is none or at best very weak pre-mating isolation between these two species. Through a novel test protocol that allows us to accurately quantify sperm production, we compared fertility among F1 males from both reciprocal crosses within species and between species. The F1 male from Okinawan *D. albomicans* ♀ x Indian *D. nasuta* ♂ produced very few sperm, just about 5% of that by the reciprocal male, who produced significantly more sperm than the intraspecific control. We also found gross abnormality in spermatogenesis accompanying sperm reduction. The concurrence of sex-ratio meiotic drive and hybrid male sterility suggests a possible causal link in between, consistent with the theory that genomic conflict in general, sex-ratio meiotic drive in particular, is a predominant evolutionary force in speciation.

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Tables and Figures

Introduction

Species is defined as the basic evolutionary unit, where evolutionary processes occur independently of other such units, among which lack of gene flow is required to establish the species status. Historically, there have been many different definitions for species, many of which emphasize one or several aspects of the biological entity that is defined as a species (De Queiroz 2007). From a perspective of species formation and establishment, the most pertinent definition of species is the biological species concept (BSC)(Dobzhansky 1937; Mayr 1942). BSC clearly stresses the genetic processes that are involved in speciation, particularly, the means by which gene flow is blocked among newly formed species. Dobzhansky (1937) classified the mechanisms of reproductive isolation including two broad categories: prezygotic and postzygotic. Mayr (1942) further identified two modes of speciation: allopatric and sympatric. In the allopatric mode of speciation, a geographic isolation necessarily predates speciation, which happens after the formation of geographic barriers to gene flow among populations previously belonged to the same species. In the sympatric mode of speciation, new species emerges without preformed barriers to gene flow; rather speciation is an active adaptive evolutionary process under the constant pressure of gene flow. Mayr (1942) argued that the allopatric mode is the *modus operandi* in speciation and the sympatric speciation is theoretically difficult. Regardless of the speciation mode, understanding the mechanisms of reproductive isolation and their underlying genetics has been the focus of speciation study in the past half century.

Prezygotic isolation mechanisms include all those that prevent the formation of zygote such as ecological, behavioral or mechanical barriers to mating, or physiological or biochemical reactions that prevent sperm-egg fusion (Dobzhansky, 1937). Especially, courtship is species specific. When a pair of flies is encountered in the field, they exchange acoustic, visual, and olfactory signals and distinguish between conspecifics and heterospecifics (Ehrman and Kim 1997; Greenspan and Ferveur 2000). Since hybrid formation is reproductively wasteful due to its sterility or inviability, such prezygotic isolation mechanisms are more efficient in preventing hybrid formation and are favored by natural selection, *i.e*., reinforcement.

Postzygotic isolation mechanisms include all those that prevent the interspecific hybrids from being reproductively successful, such as hybrid lethality, hybrid sterility or hybrid breakdown in the later generations of interspecific crosses (Dobzhansky 1937). The genetic investigations of these hybrid problems have a long history since Darwin but has been making great progresses in the last two decades thanks to the molecular biology revolution (Coyne 1992). It is generally agreed that the hybrid problems are caused by the genetic incompatibility of genes from different species. These so called Dobzhansky-Muller (D-M) factors has been under adaptive selection and has the optimal fitness in their own genome; however, they will become ill-adaptive if exposed to an alien genomic environment such as in hybrids (Turelli and Orr 2000). The holly grail of speciation studies is to identify these D-M factors and understand how they have been evolving to cause speciation.

In Drosophila, two generalizations have been drawn about D-M factors. First, D-M factors have been accumulated at least one order faster for hybrid male sterility than hybrid female sterility or hybrid lethality (Tao and Hartl 2003). Second, they have a two to four-fold enrichment on the X chromosomes than on the autosomes (Tao et al. 2003; Masly and Presgraves 2007). These two patterns are named “faster male” and “large X effect”, respectively. One evolutionary theory based on genetic conflicts might provide explanation for these two general patterns (Meiklejohn and Tao 2010).

Evolutionary theorists noted long time ago that sex-linked and autosomal genes are not equal in terms of genetic transmission (Hamilton 1967). For example, genes on the X can gain advantages on the expense of the Y-linked genes if the X-carrying father can sire more daughters than sons, while autosomal genes would always prefer equal sex ratio (Fisher 1930). This phenomenon of non-Mendelian segregation is called meiotic drive (Sandler and Novitski 1957). Consistent with this notion, numerous cases of X-linked mutations that cause female-biased sex ratio (sex-ratio meiotic drive or SR) have been discovered in various species of Drosophila (Jaenike 2001), even being molecularly identified (Tao et al. 2007a; Tao et al. 2007b). Two decades ago, the possible role played by sex-ratio meiotic drive has been speculated (Frank 1991; Hurst and Pomiankowski 1991). The idea was simple: If sex-ratio meiotic drive happens all the time, the genes in the genome other than on the X must be selected to control the driver’s selfish behavior, thus perpetually modifying the molecular machinery for male meiosis. Therefore, the male meiosis must have a fast pace in evolution, thus leading to the “faster male” evolution. Because the genetic conflict is largely between the X and

the rest of the genome, the X must have a disproportionate share of the total evolutionary change, thus leading to the “large X effect” (Meiklejohn and Tao 2010).

The genetics of both prezygotic and postzygotic mechanisms have been intensively studied in the past 70 years since the New Synthesis (Coyne and Orr 2004). However, hardly any simultaneous studies of both prezygotic and postzygotic isolation have been reported except a few exceptions, for example, one in *Mimulus* (Ramsey et al. 2003). Different authors might emphasize from their own vintage point one or the other isolation mechanism as the major cause for speciation, an objective and unbiased comparison would be extremely informative to identify the really important mechanism, thus allowing us to elucidate the essential genetic and evolutionary cause for speciation. Particularly, I would ask whether meiotic drive is a predominant or even the most important evolutionary mechanism for speciation.

Two fruit fly species, *D. nasuta* and *D. albomicans*, are closely related species. *D. nasuta* is found in Kenya, Madagascar, Seychelles, Mauritius, Sri Lanka and India, while *D. albomicans* is found in Okinawa of Japan, Southern China, Indochina, Thailand and East India (Figure 1). So far there is no evidence for their geographically overlapping, so their distribution is allopatric (Kitagawa et al. 1982). They are morphologically indistinguishable, but they have different karyotypes. In *D. albomicans*, the X and the 3rd chromosome are fused to form the neo-X, and the Y and the 3rd chromosome are fused to form the neo-Y (Figure 2). *D. nasuta* and *D. albomicans* are evidently in the incipient stage of speciation. Previous studies have inconsistent reports on their pre-zygotic isolation (Chang and Ayala 1989; Tanuja et al. 2001; Chang and Tai

2007), but no post-zygotic isolation has been reported so far for these two species. In the lab, sex-ratio meiotic drive was observed in the F1 males (SR males) from the cross between Okinawan *D. albomicans* ♀ and Indian *D. nasuta* ♂, while males from the reciprocal cross (NSR males) sired offspring of normal sex-ratio (Yang 2004).

Here I study two fruit fly (*Drosophila*) to investigate the role of meiotic drive in speciation. I will first re-assess the issue of pre-mating isolation and meanwhile investigate whether there is any significant level of post-zygotic isolation between *D. albomicans* and *D. nasuta*. Because pre- and post-zygotic isolations are studied by the same person and in the same lab with the same strains, I can obtain an objective comparison of the relative strengths of the pre- and the post-zygotic isolations between these two species.

Materials and Methods

1. *Drosophila* stocks

Outbred stocks:

D. albomicans –

MYH: E-10802/MYH01-05, Miyakojima, Okinawa, Japan, 2001

SHL: E-10815/SHL48, Shilong, India, 1981

IR: E-10806/IR96-13, Iriomotejima, Okinawa, Japan, 1996

KM: E-10811/KM01-5, Kumejima, Oknawa, Japan, 2001

D. nasuta –

M: G86, Mauritius, 1979

C: 15112-1781.13,Yaounde, Cameroon, 2004

K: 15112-1781.06, Mombasa, Kenya, 1976.

Other than the last two stocks obtained from the Drosophila Species Stock Center (UC San Diego), all the others were obtained from Dr. Masayoshi Watada, Ehime University, Japan.

Inbred Stocks:

Three inbred lines were constructed by sib pair matings for 15 generations, as listed below:

D. albomicans strain ALB2 - from the strain MYH

D. albomicans strain SHL1 and SHL2 - from the strain SHL

D. nasuta strain NAS3 - from the strain M.

2. Premating behavior

a. Courtship behavior

No-choice test was used to analyze courtship behavior between a female and a male in four different combinations (Kim and Ehrman 1998): ALB2 x ALB2, ALB2 x NAS3, NAS3 x ALB2, NAS3 x NAS3. For each observation a female and a male were

introduced without anesthetization in to a small glass vial (1.5-cm width X1.0-cm height). One pair of flies were introduced to small mating chambers and courtship behavior between them was observed for 10 minutes. The characteristic courtship behaviors of fruit fly, including orientation, tapping, circling, wing vibration, licking and copulation attempt, were recorded using a JVC camcorder. During the 10 min interval, courtship latency and courtship duration were measured. I also observed courtship behavior of non-inbred strains to see whether there are any differences between inbred and non-inbred flies. Courtship data was statistically analyzed using a SAS/JMP statistics software and non-parametric two-factor ANOVA was performed.

b. Mating Behavior

Multiple choice test was used to determine sexual isolation between *D. albomicans* and *D. nausta* using non-inbred flies. Twelve pairs of 7 day old virgin flies from each of the two strains were introduced into a single mating chamber. Since two species are morphologically identical, one wing of one species was minimally notched so that mating pairs could be identified. Four replicates were observed for each combination of species. In two replicates, the wings of *D. albomicans* males and females were notched, and in the other two, the wings of *D. nasuta* males and females were notched. Notching was not found to affect male activity during courtship or mating, neither it was found to affect female discrimination of mates. Observation was carried out at 22 ˚C and all matings were recorded for 1 hour.

c. Statistical Analysis

The joint isolation index (*I*) has been widely used to measure extent of sexual isolation in the multiple choice tests. However, a major criticism of the joint isolation index (*I*) is its poor statistical estimation properties due to uncorrected marginal effects (Rolán-Alvarez and Caballero 2000). A new index, I_{PSI}, was designed to incorporate the estimates (*PSI*) of mate choice coefficients for each type of mating pair. It was later demonstrated that I_{PSI} considerably reduces the statistical bias of the estimates and has several statistical advantages over *I* while retaining the advantage of a simple relationship to the frequencies of homogamic and heterogamic matings (Pérez-Figueroa et al. 2005). Therefore, I utilized I_{PSI}, using the JMATING software developed for the analysis of mating frequency data (Carvajal-Rodriguez and Rolán-Alvarez 2006). JMATING resamples 10,000 times the observed values of mating pairs in order to estimate the bootstrap sampling distribution for the estimator (I_{PSI}). Then the program calculates the bootstrap average and standard deviation as well as the two-tail probability of getting a sexual isolation estimate significantly different from zero, which is equivalent to random mating.

3. Quantifying F1 hybrid fertility

a. A novel protocol

The following types of F1 males were produced from crosses of 5-10 pairs of flies, aged in different vials for 4-5 days. The vials holding the females were checked a few days later for crawling larvae to exclude any non-virgin females. Six types of F1 males were produced and used for fertility quantification. The experiments for C and D males are

still under going at this moment, so I will report the results only from A, B, E and F males.

A: ALB2 ♀x NAS3 ♂

B: NAS3 ♀x ALB2 ♂

C: SHL2 ♀x NAS3 ♂

D: NAS3 ♀x SHL2 ♂

E: ALB2 ♀x SHL2 ♂

F: SHL2 ♀x ALB2 ♂

For each pair of F1 males, I used the following stocks for the tester virgin females:

A and B: ALB2

C and D: NAS3

E and F: ALB2

I designed an accurate albeit tedious protocol for the purpose of quantifying male fertility (Figure 3). The rationale is to count offspring of males by allowing each male to mate with excess number of females and thus exhausting his sperm. The protocol was based on extensive pilot trials. The six types of males were tested as pairs (A and B, C and D, E and F). Thirty freshly eclosed males of each type were collected at the beginning of the experiment.

Day 1: Each male was placed with three mature virgin females (5 day old) in a vial.

Day 2: The male was picked up under slight CO2 anesthetization and transferred to a fresh vial with 3 mature females. The three females from the first vial were put back.

Day 3: The same as Day 2.

Days 4-7: The male was transferred to a vial with 12 mature females.

Day 8: The male was transferred to a vial with 3 mature females.

Days 9-12: The same as Days 4-7.

Day 13: The same as Day 8.

….

The above protocol was continued until the male died or became sterile. For each vial of one day mating (Day 1, 2, 3, 8, 13 etc.), the three females were allowed to lay eggs for four days and then were transferred to the second vial on the 4^{th} day and again to the third vial on the 10^{th} day. Offspring from the three females would not grow in crowding condition. Occasionally, if abundant larvae were observed in the third vial on the 17^{th} day, the females were transferred to the fourth vial. All offspring were sexed and counted. Usually, only a few offspring were found in the third or fourth vial. Offspring from the vials of 4 day mating (Days 4-7, 9-12 etc) were not counted.

By this protocol, every single sperm from the tested male can fertilize an egg and the offspring count is assumed to be a close proximate to the sperm count. By sampling once every 5 days, I cut 80% of the labor.

b. Statistics

All or nearly all males (A, B, E and F) were fertile across their lifespan, but not necessarily producing any offspring in a particular mating day. The only one sterile male (of the A male type) for his whole life was excluded from statistics. I calculated the population-wise average offspring for each mating day, regardless some males did not sire any offspring on that particular day. To obtain the total sperm count, I estimated the offspring produced in a 4-day mating period by interpolating from the sperm counts obtained from the two flanking one-day matings. For example, the sperm produced in Days 4-7 is the mean of Day 3 and Day8 times four. The cumulative sperm count on a particular day was the sum of all sperm produced prior to that time.

4. Ultrastructural study of spermatogenesis through TEM

In conjunction with the above sperm quantification, we also made TEM observations of spermatogenesis in the A and B males. Due to time constraint, C-F males will be examined in the coming weeks. Testes and accessory glands were dissected from young males (2-3 d old) with a fine tungsten needle and were transferred immediately to 2% glutaraldehyde in 0.067M phosphate buffer on ice. The specimens was fixed for 2 h at 4˚C in 1% paraformaldehyde and 2% glutaraldehyde in 0.067 M phosphate buffer, followed by a post fixation of 1h in 2% OsO4 at 4˚C. The specimen was treated with 1 % uranyl acetate at room temperature and then trimmed after ethanol dehydration so that only one of each pair of testes was used in embedding.

Each testis were cut into 4-5 segments and aligned on the bottom of the mold in a straight line with the apical tip facing out. Sections were cut on a Reichert ultracut-S microtome, followed by staining with uranyl acetate and lead citrate. The grids were observed with HITACHI H-7500 electron microscope.

5. Polytene chromosome maps of ALB2 and NAS3

To obtain preliminary data for future genetic mapping, I assessed whether the two strains, ALB2 and NAS3, are homosequential by observing their polytene chromosomes. Third instar larvae from ALB2 and NAS3 were dissected in 35% acetic acid. The salivary gland was then transferred into 2% Orcein/acetic acid (w/v) on siliconized slides. The salivary gland was stained for 3-5 min, squashed, and observed under a compound microscope. The images of individual chromosomes or segments of chromosome were taken and processed using the software Photoshop. This work is still in progress and I will not present it here.

Results

1. There is very weak premating isolation between *D. albomicans* and *D. nasuta*

a. Courtship behavior

Courtship index (CI) was measured by dividing a total duration of six male courtship elements by 10. I also measured courtship latency (CL), the time when a male displayed its first courtship toward a female after they were introduced to the

observation chamber. Figure 4A shows courtship indices for four different mating combinations between two species in inbred and outbred lines, respectively. There is no significant difference in courtship indices between inbred and outbred lines ($p = 0.0516$). Other than a single case of significantly higher CI of outbred lines (the A x N combination), there is no overall higher CI between conspecific combinations and heterospecific ones ($p = 0.7616$).

Similar to CI, courtship latency (CL) among four different mating combinations between two species using inbred and outbred lines was also measured (Figure 4B). Inbred lines appeared to show longer CL than outbred lines in heterospecific matings (A x N and N x A), though not statistically significant ($p = 0.0588$). Only the N x A combination showed a significant difference in CL between inbred and outbred lines. Overall, there was no significant difference in CLs among the eight different mating combinations ($p = 0.1489$).

b. Mating behavior

Using multiple choice tests in the mating chambers, I observed homogamic matings and heterogamic matings between *D. albomicans* and *D. nasuta*. Four *D. albomicans* strains (MYH, KM, IR and SHL) and three *D. nasuta* strains (Cameroon, Mauritius and Kenya) were tested. I calculated I_{PSI} using the JMATING software (Carvajal-Rodriguez and Rolán-Alvarez 2006). Table 1 shows the results of mating behavior between *D.albomicans* and *D. nasuta*. I found that two species randomly mated with each other. Among eight mating combinations, the isolation indices (I_{PSI}) showed no significant difference from random mating. One pair of species (SHL and Mauritius) showed significant sexual isolation. However, there was no significant sexual isolation observed

between any of the Japanese *D. albomicans* strains (MYH, KM and IR) and either of the Cameroon and Kenya *D. nasuta* strains.

2. Male fertility is severely reduced in the F1 male from the cross ALB2 ♀ x NAS3 ♂ but not in the F1 male from the reciprocal cross.

Using the "sperm exhausting" mating protocol (Figure 3), I essentially "counted" the sperm produced by A, B, E and F males. The mating test for the E and F males are still in progress, so all comparisons are made on the 33rd day. The cumulative sperm count per male is estimated for the four types of males (see Materials and Methods). On day 33, the total sperm count of the A male is obviously much lower than the others, just about 5.7% of the B males (t-test, $p = 3x10^{-20}$). The B male, though being F1 from interspecific cross, is significantly more fertile than E and F males of the intraspecific crosses (Figures 5 and 6). There is evidently heterosis in terms of male fertility in the B male. The A male sired female-biased offspring (91.1%), in contrast to all the others with roughly equal sex-ratios of 51.3%, 51.0% and 52.2% for the B, E and F males, respectively (Figure 6).

3. Ultrastructural study of spermatogenesis through TEM

Using transmission electron microscopy (TEM) I observed the ultrastructure of spermatogenesis in the A and B males. I observed gross abnormalities in the A males but normal features in the B males. Similar work for the C, D, E and F will be done in the near future.

The abnormalities of the A male can be described in several respects. First, there is high frequency of fusions between two or more spmermatid tails (axonemes) in the pre-individualization stage (Figure 7A, compared to normal morphology of the B male in Figure 7D). The nuclear condensation process of spermiogenesis in the A male appears to be normal. However, nuclei (sperm heads) within a bundle show significant variance in the positions of cross sections, suggesting gross disorganization in alignment (Figures B and E). In the basal portion of testis where mature sperm are enriched, the A male has much reduced number of normal sperm; abnormal sperm with fused tails were predominantly observed in the sperm pool (Figure 7C and F). The gross abnormalities observed by TEM for the A male is consistent with the fertility quantification described above, confirming that the A male produced 95% of the normal sperm production.

Discussion

In this study, I designed a novel test protocol to accurately quantify sperm production. I investigated pre-mating behaviors and hybrid sterility between *D. albomicans* and *D. nasuta*. I found very little of the former but very significant fertility reduction in the F1 males from the cross of Okinawan *D. albomicans* ♀ x Indian *D. nasuta* ♂, while the F1 males from the reciprocal cross have normal or even better fertility than control. In addition, I examined spermatogenesis of two types of F1 hybrids and found abnormal morphology of sperm from the SR but not from NSR males. There is a correlation between sex-ratio meiotic drive and hybrid male sterility. Put together, the evidence

collected here supports the idea that meiotic drive could be a predominant evolutionary mechanism for speciation.

Courtship behavior among the Drosophila species plays a significant role in strengthening reproductive isolation between closely related species (Spiess 1987). These behaviors function in preventing the generation of hybrids and assist in maintaining the separation of two closely related species. The implementation of species specific courtship behavior is necessary, especially when two or more species coexist sympatrically. Between *D. albomicans* and *D. nasuta*, the courtship index (CI) from the inter-specific matings is generally not different from the intra-specific matings, so is the courtship latency (CL) (Figure 4). I did, however, observed some CI and CL that suggest elevated pre-mating isolation between these two species, but all coming from inbred stocks, not from outbred stocks. So this is probably a behavioral artifact caused by the general lack of physical vigor in the inbred animals.

In the mate choice experiments, we found that two sibling species, *D. albomicans* and *D. nasuta*, randomly mated as reported before (Chang and Ayala 1989). Four geographic strains of *D. albomicans* were tested with each of three *D. nasuta* strains. The degrees of sexual isolation range from -0.10 to 0.60. Out of 11 mating combinations, 10 combinations showed no significant sexual isolation between two species. Only one combination between the SHL strain of *D. albomicans* and the Mauritius strain of *D. natura* showed strong sexual isolation. The two *D. nasuta* strains used in this study, Mauritius and Kenya, have been kept for more than 30 years in the laboratories. They probably have been highly inbred. For example, males of both the

Mauritius and Kenya strains were very weak and did not mate with females as much as *D. albomicans* males did. In contrast, males of the *D. nasuta* strain collected in Cameroon in 2006 were very active and mated as much as *D. albomicans* males.

Taken together, I found no premating isolation between *D. albomicans* and *D.nasuta*. This might be not a surprise, because their distribution in nature is allopatric. In a classic meta-analysis, strong evidence was found for the reinforcement of sexual isolation between sympatric species but not between allopatric species (Coyne and Orr 1989, 1997). Because *D. albomicans* and *D. nasuta* are allopatric, so the weak or non-existent pre-mating isolation should be expected, given the fact that these two species have only diverged recently (Bachtrog 2006).

Postzygotic isolation occurs when hybrids are lethal or unable to produce fertile offspring, such as the well-known example of mule, which is the sterile hybrid from a horse dam and a donkey sire. The F1 hybrids from both reciprocal crosses of *D. albomicans* and *D. nasuta* were reported as “fertile” because both can mate and produce abundant F2 offspring. However, when testing male fertility in a rigorous way such as the protocol used in this study, male fertility can be quantitatively measured and even a subtle difference in sperm production can be detected. Surprisingly, only about 5% of normal sperm production remains in the F1 male from the cross of *D. albomicans* female x *D.nasuta* male, but the reciprocal F1 male has even higher fertility than intra-specific controls (Figures 5 and 6).

Interestingly, the same infertile F1 male sires strongly female biased offspring, as observed before (Yang et al. 2004). If normal spermatogenesis produces equal ratio of X- and Y-carrying sperm, the sex ratio in the offspring would be 1: 1. Here, the female-biased sex ratio implies that Y-carrying sperm is only a small proportion (~10%) of the functional sperm. Under TEM, I observed a gross abnormality for most sperm (Figure 7), suggesting that most sperm, both the X- and the Y-carrying, are dead; but the X-carrying sperm survive better than the Y-carrying sperm. As in other cases of sex-ratio meiotic drive (Jaenike 2001), there must be one or several distorter genes on the X chromosome of *D. albomicans*. Meanwhile, the Y chromosome from *D. nasuta* must be liable to the destructive action of the distorter gene. Here, I observed sex-ratio meiotic drive and hybrid male sterility in the same male genotype, suggesting a possible causal link between the two.

Twenty years ago, a theory linking speciation and meiotic drive was proposed (Frank 1991; Hurst and Pomiankowski 1991), but was soon were criticized by different authors (Johnson and Wu 1992; Charlesworth et al. 1993; Coyne and Orr 1993), mainly because of a lack of empirical evidence. However, there is a surge of discoveries that indeed implicate meiotic drive as a major cause for hybrid male sterility (Tao et al. 2001; Phadnis and Orr 2009). The study reported here further garner evidence in favor of the meiotic drive cause for speciation. Particularly, a lack of premating isolation but meanwhile a strong hybrid male sterility correlated with meiotic drive argues that genetic conflict within a genome could be the predominant evolutionary mechanism for speciation.

Currently, I am continuing fertility quantification for C, D, E, and F males and at the mean time we are preparing to conduct TEM observation for these F1 males. We expect to see normal offspring production from these males and an ultrastructure of spermatogenesis in these males should reveal normal morphology. These results in conjunction with current results should provide stronger support for the casual link between meiotic drive and hybrid male sterility. Further, additional studies profiling hydrocarbons in terms of sexual dimorphism and interspecific difference and relating the hydrocarbon profiles to premating behaviors are in progress. These studies will provide further evidence for the weak pre-mating isolation between these two species.

The ultimate goal of studying meiotic drive and speciation in *D. albomicans* and *D. nasuta* is to molecularly identify the underlying genes. Genetic dissection to reach gene cloning is feasible only if the two species are homosequential (sharing the same gene order), and thus amenable to recombination mapping. To obtain preliminary data for future genetic mapping, I am assessing whether the three strains, ALB2, NAS3, and SHL2 are homosequential by observing their polytene chromosomes. In preparation of standard map of the salivary chromosomes from ALB2 and NAS3, images of individual chromosomes or segment of chromosome are taken and processed in order to digitally reconstruct the chromosomes arms. If inversions exist between strains or species, they can be easily identified in F1 hybrids. Homosequential strains or species can be therefore selected with careful observations of polytene preparations.

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Figure Legends

Figure 1. Geographic distribution of *D. nasuta* and *D.albomicans*

Figure 2. *D. albomicans* and *D. nasuta* have different karyotypes. (A) *D. nasuta* has an acrocentric X chromosome, a submetacentric Y, a pair of metacentric 2nd chromosomes, a pair of acrocentric 3rd chromosomes, and a pair of small dot-like 4th chromosomes (Wakahama et al. 1983). (B) In *D. albomicans,* there is a fusion of the large acroccentric 3rd chromosome with the X and Y (Chang et al. 2008).

Figure 3. The protocol for quantifying male fertility

Figure 4. (A) Courtship index (CI), and (B) Courtship Latency (CL) measured for the eight different combinations between *D. albomicans* (A) and *D.nasuta* (N). Each combination is set up in the direction of (♀x ♂).

Figure 5. The average number of offspring sired by different types of males. The experiments for the E and F males are still undergoing, so the counts for these them are not shown after day 38 and 33, respectively.

Figure 6. The cumulative offspring produced by the A, B, E and F males during their whole life span and their sex-ratio in female %. The counting for E and F are still undergoing.

Figure 7. Spermatogenesis in the A male (A-C) and B male (D-F) as observed under TEM. (A, D): Cross section of spermatid tails from well developed but pre-individualization bundle. (B, E): Cross section across nuclei at the stage of condensation. (C, F): Cross section across the basal portion of testis full of mature sperm.

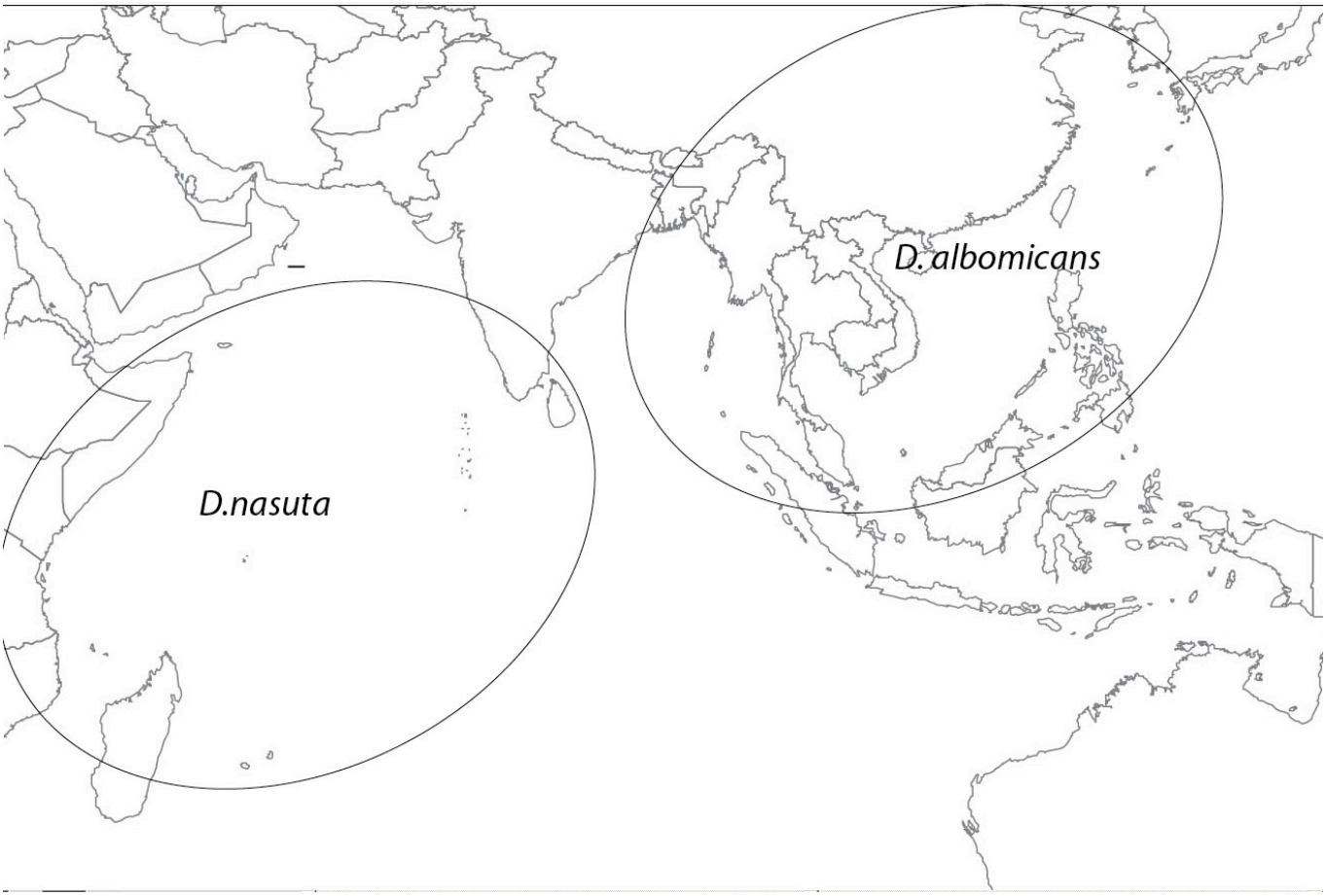
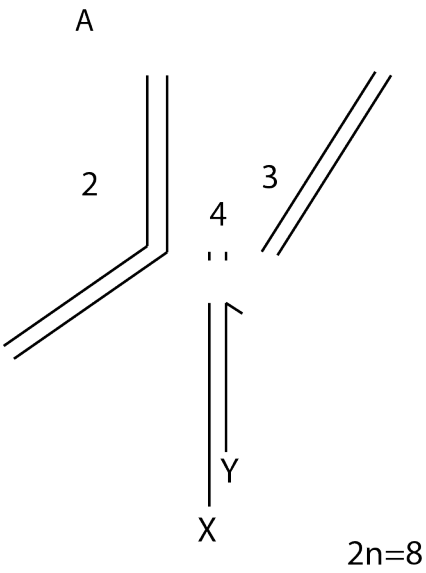
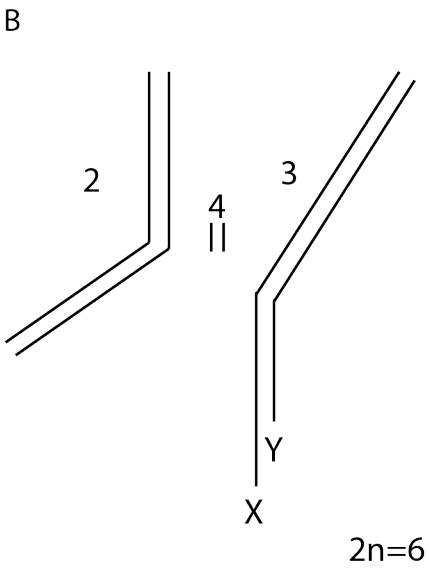


Figure 1



D.nasuta



D.albomicans

Figure 2

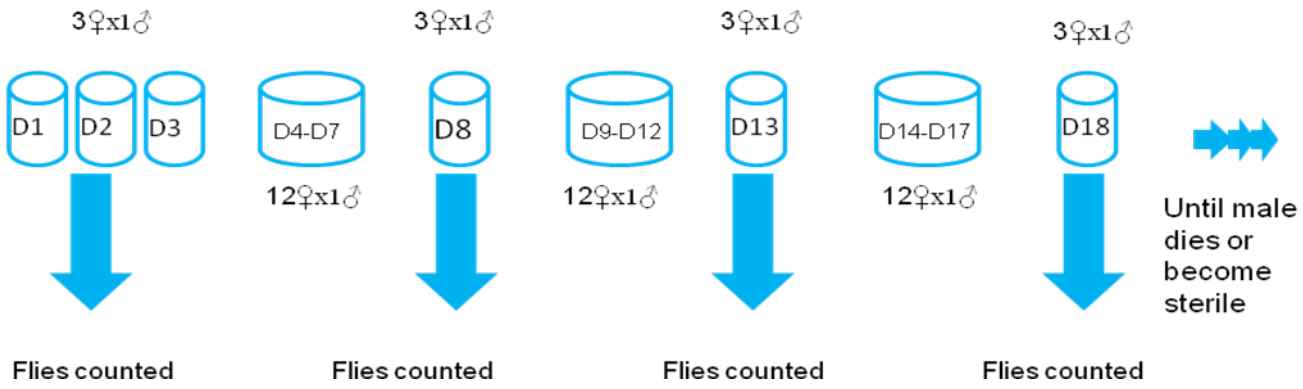

3♀x1♂
D1
D2
D3
Flies counted
D4-D7
12♀x1♂
3♀x1♂
D8
Flies counted
D9-D12
12♀x1♂
3♀x1♂
D13
Flies counted
D14-D17
12♀x1♂
3♀x1♂
D18
Flies counted
Until male dies or become sterile

Figure 3

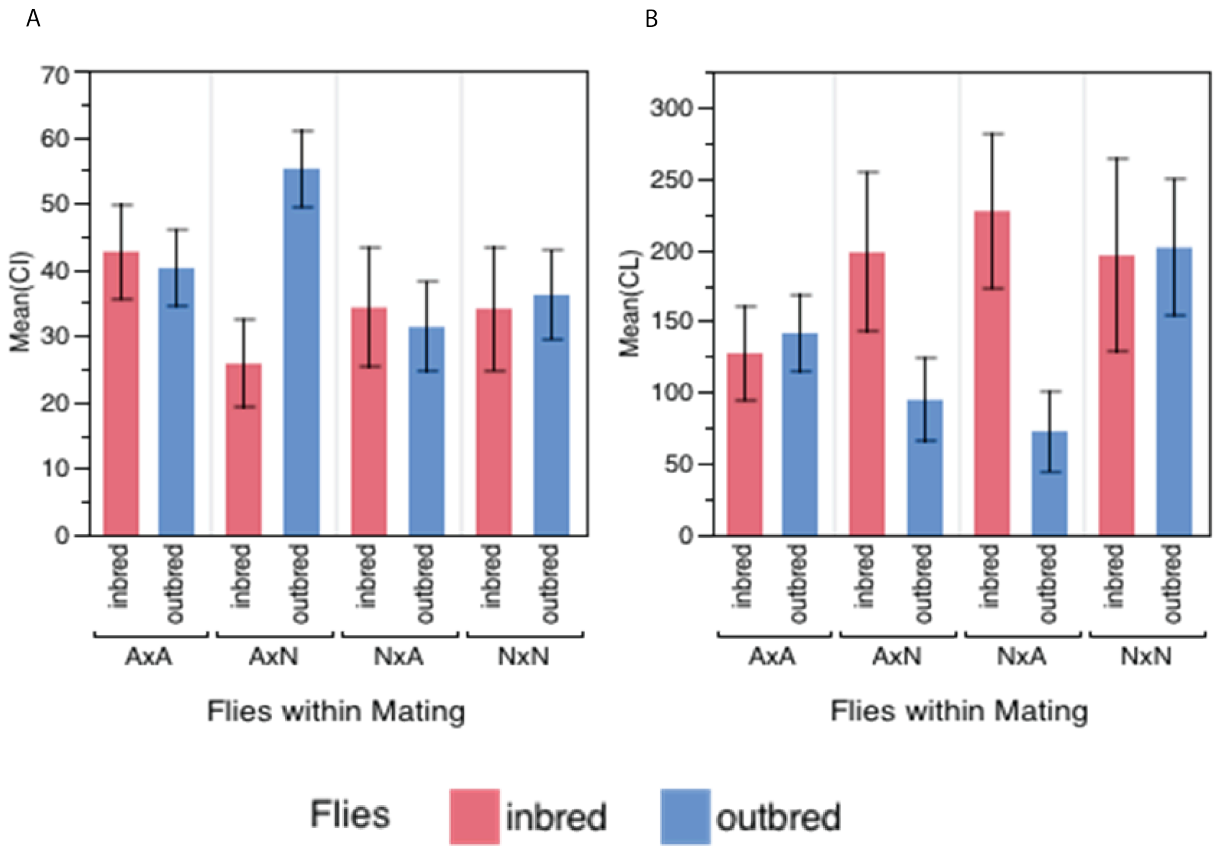

A
B
Mean(CI)
Mean(CL)
70
60
50
40
30
20
10
0
300
250
200
150
100
50
0
inbred
outbred
AxA
AxN
NxA
NxN
Flies within Mating
Flies
inbred
outbred

Figure 4

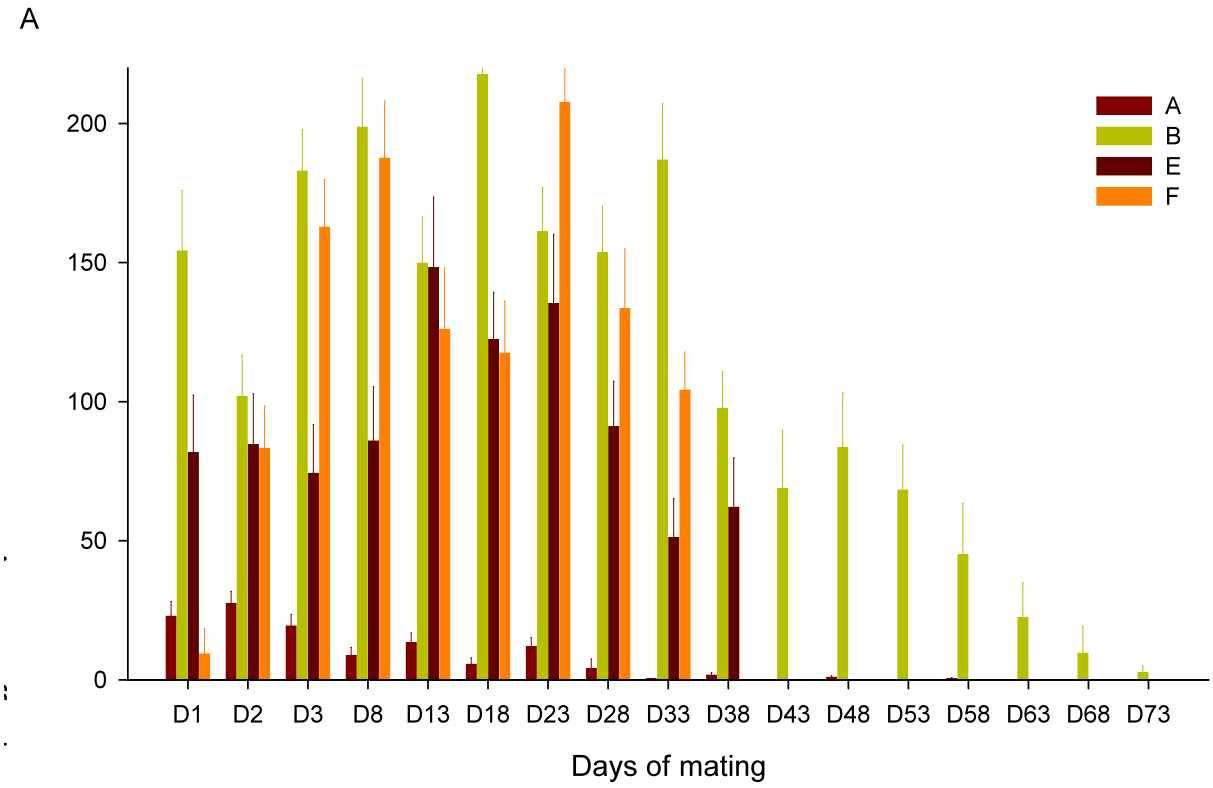

A
200
150
100
50
0
A
B
E
F
D1
D2
D3
D8
D13
D18
D23
D28
D33
D38
D43
D48
D53
D58
D63
D68
D73
Days of mating

Figure 5

t-test

	A	B	E
B	3.392E-20		
E	6.098E-07	1.336E-4	
F	1.120E-11	3.984E-2	4.812E-2

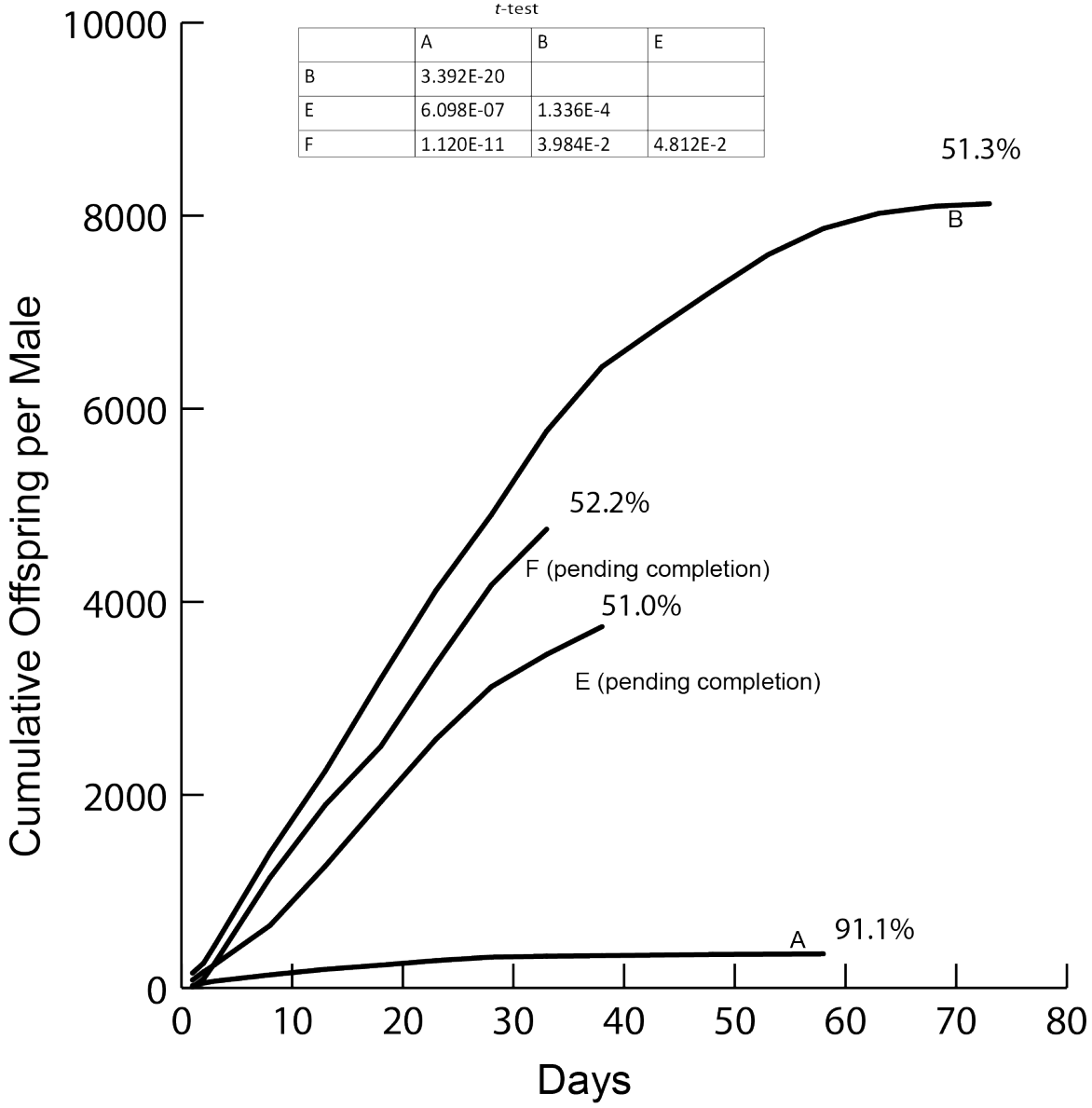


Figure 6

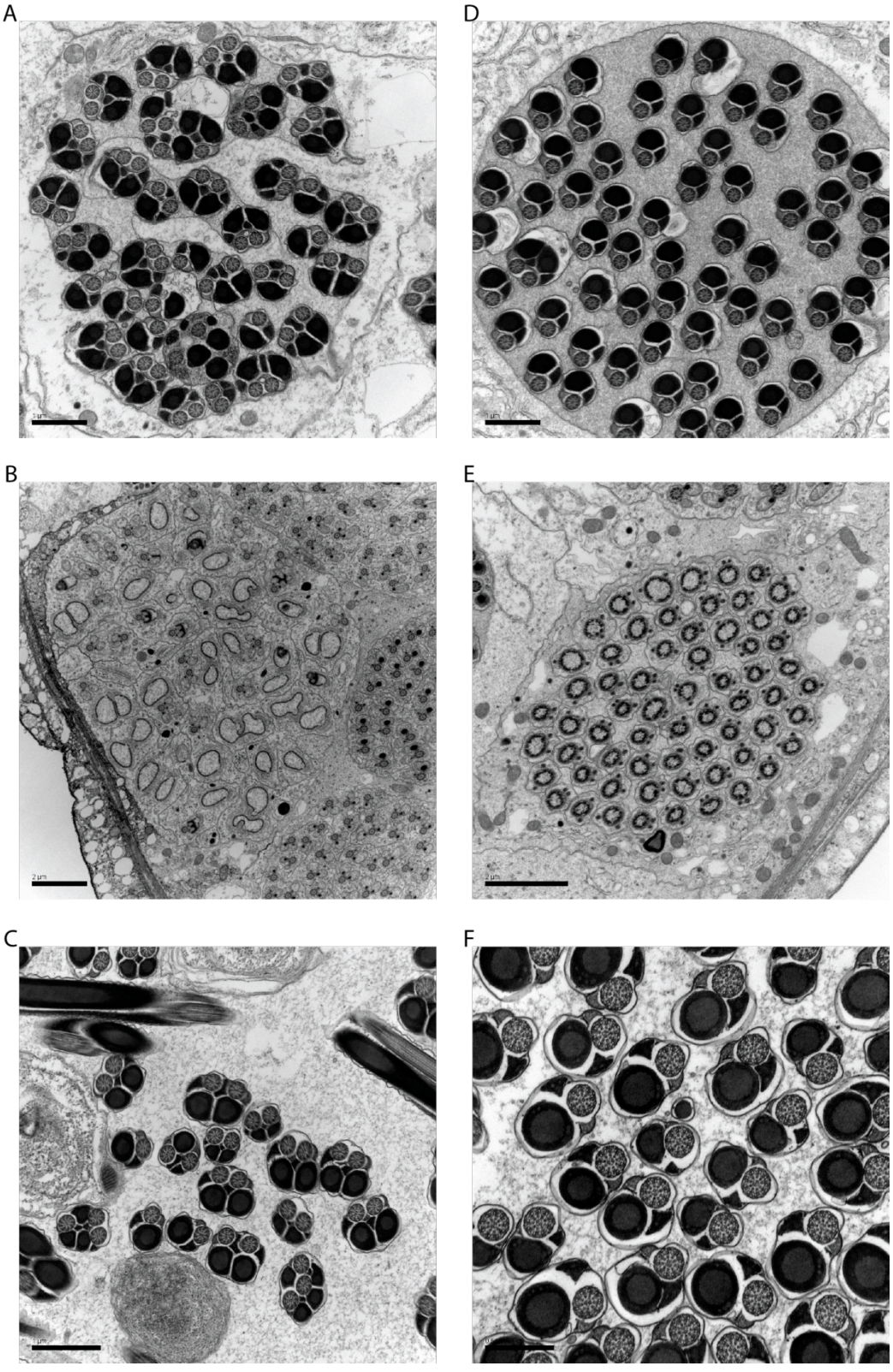


Figure 7

Table 1. Premating isolation in the multiple choice tests between *D. albomicans* and *D. nasuta*

Strain A x Strain B	#matings	$A_{♀}$ x $A_{♂}$	$A_{♀}$ x $N_{♂}$	$N_{♀}$ x $A_{♂}$	$N_{♀}$x $N_{♂}$	I_{psi} ± SD	t test	p
MYH x M	82	28	16	17	21	0.15±0.11	1.38	>0.05
KM x M	68	35	6	24	3	-0.08±0.12	-0.64	>0.05
IR x M	57	28	5	16	8	0.25±0.13	1.97	>0.05
SHL x M	39	22	6	2	9	0.60±0.13	4.68	<0.05
MYH x C	51	8	21	8	14	-0.10±0.14	0.73	>0.05
KM x C	62	25	11	16	10	0.27±0.13	0.69	>0.05
IR x C	51	14	12	7	18	0.25±0.13	1.98	>0.05
SHL x C	61	16	15	9	21	0.22±0.12	1.79	>0.05
MYH x K	51	18	15	10	8	-0.01±0.14	-0.07	>0.05
KM x K	39	19	6	10	4	0.23±0.15	1.55	>0.05
SHL x K	38	19	10	6	3	-0.01±0.16	-0.08	>0.05

MYH = Miyakojima, Japan; KM = Kumejima, Japan; IR = Iriomotejima, Japan; SHL = Shilong, India; M = Mauritius; C = Cameroon; K = Kenya.