A Neo-Sex-Chromosome that Drives Post-Zygotic Sex Determination in the Hessian fly (*Mayetiola destructor*)

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ABSTRACT

Two non-overlapping autosomal inversions defined unusual neo-sex-chromosomes in the Hessian fly (*Mayetiola destructor*). Like other neo-sex-chromosomes, these were normally heterozygous, present only in one sex, and suppressed recombination around a sex-determining master switch. Their unusual properties originated from the anomalous Hessian fly sex determination system in which post-zygotic chromosome elimination is used to establish the sex-determining karyotypes. This system permitted the evolution of a master switch (*Chromosome maintenance, Cm*) that acts maternally. All of the offspring of females that carry *Cm*-associated neo-sex-chromosomes attain a female-determining somatic karyotype and develop as females. Thus, the chromosomes act as maternal effect neo-Ws, or W-prime (W’) chromosomes, where ZW’ females mate with ZZ males to engender female-producing (ZW’) and male-producing (ZZ) females in equal numbers. Genetic mapping and physical mapping identified the inversions. Their distribution was determined in nine populations. Experimental matings established the association of the inversions with *Cm* and measured their recombination suppression. The inversions are the functional equivalent of the sciarid X-prime chromosomes. We speculate that W’ chromosomes exist in a variety of species that produce unisexual broods.
SEX CHROMOSOMES are usually classified as X, Y, Z, or W based on their pattern of segregation and the gender of the heterogametic sex (OHNO 1967). However, when chromosome-based sex determination occurs post-zygotically, the same nomenclature confounds important distinctions, and may hide interesting evolutionary phenomena. The Hessian fly (Mayetiola destructor), a gall midge (Diptera: Cecidomyiidae) and an important insect pest of wheat, presents an excellent example (STUART and HATCHETT 1988; STUART and HATCHETT 1991). In this insect, all of the female gametes and all of the male gametes have the same number of X chromosomes (Figure 1A); no heterogametic sex exists. Nevertheless, Hessian fly sex determination is chromosome based; post-zygotic chromosome elimination produces different X chromosome to autosome ratios in male (A1A2X1X2/A1A2OO) and female (A1A2X1X2/A1A2X1X2) somatic cells (where A1 and A2 are the autosomes, X1 and X2 are the X chromosomes, and the paternally derived chromosomes follow the slash) (MARIN and BAKER 1998; STUART and HATCHETT 1991). Thus, Hessian fly “X” chromosomes are defined by their haploid condition in males, rather than by their segregation in the gametes.

An autosomal dominant genetic factor called Chromosome maintenance (Cm) complicates Hessian fly sex determination further (STUART and HATCHETT 1991). Cm has a maternal effect that acts upstream of X chromosome elimination during embryogenesis (Figure 1A). It prevents X chromosome elimination so that all of the offspring of Cm-bearing mothers obtain a female determining karyotype. Cm-bearing females are, therefore, monogenic and thelygenic; they produce only female offspring. The absence of Cm usually has the opposite effect; all of the offspring of most Cm-lacking females obtain a
male determining karyotype. These *Cm*-lacking females are monogenic and arrhenogenic; they produce only male offspring. Like a sex-determining master switch, *Cm* is usually heterozygous and present in only one sex (Figure 1B). Thus, thelygenic females (*Cm/-*) are “heterogametic,” as their *Cm*-containing gametes and *Cm*-lacking gametes produce thelygenic (*Cm/-*) and arrhenogenic (-/-) females in a 1:1 ratio. Some Hessian fly populations also contain amphygenic females, which produce broods containing both sexes. No mating barrier between monogenic and amphygenic families exists (Figure 1C), but amphygenic females have always been found in lower abundance (GALLUN et al. 1961; PAINTER 1930; STUART and HATCHETT 1991). In experimental matings, the inheritance of maternal phenotype was consistent with the segregation of three *Cm* alleles (Figure 1C): a dominant thelygenic allele, a hypomorphic amphygenic allele, and a null arrhenogenic allele (STUART and HATCHETT 1991).

Here we report the genetic and physical mapping of *Cm* on Hessian fly autosome 1 (A1). Two non-overlapping inversions were identified that segregated perfectly with *Cm*. The most distal inversion was present in all thelygenic females examined. The more proximal inversion extended recombination suppression. These observations suggested that successive inversions evolved to suppress recombination around *Cm* after it arose. The inversions therefore appear to have evolved in response to the forces that shaped vertebrate *Y* and *W* chromosomes (CARVALHO and CLARK 2005; CHARLESWORTH 1996; GRAVES and SHETTY 2001; RICE and CHIPPINDALE 2001). We therefore believe the inversion-bearing chromosomes may be classified as maternal effect neo-Ws. We speculate that additional prime chromosomes will be discovered in the genomes of other species that have a life history that permits maternal genotype to influence sex determination.
MATERIALS AND METHODS

**Hessian fly populations:** Hessian fly strains were maintained and experimental matings were performed as previously described (BEHURA et al. 2004). Because Cm’s effect is maternal, the Cm genotype of each female is manifested as the phenotype (sex) of her offspring. Therefore, individual females were genotyped as thelygenic (Cm/-), arrhenogenic (-/-), or amphygenic by sexing their offspring. Individual females were first permitted to mate and deposit their eggs on ‘Blueboy’ wheat seedlings in separate caged pots. In genetic mapping experiments, females were collected separately after oviposition and their DNA was extracted for molecular genotyping. The offspring of these females were allowed to develop and sexed as adults. When examining the offspring of females for the presence of inversions, chromosome preparations were made of at least 10 offspring of each female. These offspring were sexed cytologically. The remaining offspring were allowed to develop in the caged pots and sexed as adults.

**Molecular genetics:** DNA was extracted from individuals using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacture’s recommendations. AFLP-PCR fingerprinting of individual Hessian flies was performed as previously described (BEHURA et al. 2004) using the AFLP-based Genome Typing Kit for small plant genomes (Invitrogen; Carlsbad, CA). The AFLP band that co-segregated with the thelygenic Hessian fly genotype was amplified using the selective primers EcoRI-AC and MseI-CTG. To determine the DNA sequences of that AFLP band, it was excised from an acrylamide gel and cloned into the pCRII TOPO cloning vector (Invitrogen; Carlsbad, CA). Seven
plasmids containing the band were then sequenced using a BigDye Terminator Sequencing kit (Applied Biosystems; Carlsbad, CA) and an ABI 3730 DNA analyzer.

To determine recombination frequencies along the long arm of chromosome A1, individual Hessian flies were genotyped for alleles segregating for three sequenced tagged sites (STSs) and two simple sequence repeat (SSR) markers (Figure S1) that had been discovered previously in BAC sequence data (AGGARWAL et al. 2009; SCHEMERHORN et al. 2009). Each individual was genotyped using its DNA as template in separate polymerase chain reactions (PCR). Primer pairs used to amplify each marker were as follows: SSR 71 (GenBank Acc. No. EU340414), GAAGCCATTGCTCTCTCAGG and ATACGGCAAATCGAAGCATT; BAC-end STS CL27b3b (GenBank Trace Archive 2136886008), TCGTGAGTTTTTACTTGTATG and GTATCTTCGTCGTAACTCATC; BAC-end STS 4e18g (GenBank Trace Archive 2136867722), CGCTTGCTCAGGTTGTG and CGAACACTGTGGAAATGC; SSR 113 (GenBank Acc. No. GQ257854), CCTGAATATGTGCACCGGATA and TGGGAATTTTCTGTTTTTCA; and BAC-end STS 3L9b (GenBank Trace Archive 2136867352), CGAAGACATAAATATCCACG and GTGGATTTGGAGCGAAGC. PCR was performed in 15 µL containing 10 mM Tris-Cl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 µM each of the four dNTPs, 200 nM of each primer, 5 ng of genomic DNA and 2.0 units of Taq DNA polymerase. Each PCR was performed for 35 cycles of 94° for 30 s, 52° for 30 s, and 72° for 1 min and given a final extension at 72° for 10 min. The alleles of all markers, except SSR 71, were visually scored using ethidium bromide staining after 2% agarose gel electrophoresis (Figure 1S).
Marker SSR 71 was scored using a Beckman Coulter CEQ 8000 DNA analyzer according to the manufacture’s recommendations (Figure 1S).

**Molecular cytogenetics:** BAC library screening and polytene chromosome preparations were performed as previously described (BEHURA et al. 2004). BAC clones were derived from three BAC libraries, the Hf library (LIU et al. 2004a), the Mde library (BEHURA et al. 2004), and the CL library (AGGARWAL et al. 2009). Fluorescence *in situ* hybridization (FISH) to Hessian fly polytene chromosomes was performed using BAC DNA probes. BAC DNA (1 µg) was nick translated with biotin (Roche), digoxigenin (Roche), or dinitrophenyl-11-dUTP (DNP, Invitrogen). Hybridizations were performed using 20 ng of each labeled BAC per polytene chromosome preparation. Biotin-labeled probes were detected using AlexaFluor 488-conjugated streptavidin (Molecular Probes). Digoxigenin-labeled probes were detected using anti-digoxigenin rhodamine (Roche). DNP-labeled probes were detected using rabbit anti-dinitrophenyl KLH IgG (Molecular Probes) followed by chicken anti-rabbit IgG conjugated with AlexaFluor 647 (Molecular Probes). Microscopy was performed using a Nikon Eclipse 80i epifluorescence microscope. Images were captured with a Photometrics Cool Snap HG CCD camera controlled with Metamorph software (Universal Imaging Corporation).

To characterize rearrangements and order contigs on the A1 chromosome, a series of FISH experiments were performed in which a single BAC from two or three different contigs were used as probes. Digital images from these experiments were then examined to determine BAC (contig) order. To determine the orientation of a contig on the chromosome, two BACs from each of the 74 A1 contigs were used as probes in separate
FISH experiments. To detect inversions in Hessian fly populations, BACs Mde37d21 and Hf5d5 were used as probes. To estimate the length of the inversions, the relative physical length of each inversion, compared to the total length of all chromosomes, was determined in 10 images using the Metamorph software. Those estimates were then multiplied by the size of the Hessian fly genome (158 Mb) and the means and standard errors were calculated.

RESULTS

Genetic and physical mapping of Cm: To identify a Cm-linked DNA marker, we genotyped females in a single Indiana L strain family as thelygenic (n=35) or arrhenogenic (n=48) and then subjected the DNA of each genotyped female to AFLP-PCR DNA fingerprinting. One AFLP band that failed to recombine with the thelygenic phenotype was discovered (Figure S1). Upon determining the sequence of this band, we recognized that it was identical to marker 2709 (GenBank accession number BV079629), an AFLP that had been mapped previously within a cluster of 12 AFLPs near the telomere of the long arm of Hessian fly polytene chromosome A1 (BEHURA et al. 2004). In that study, we noted the long arm of A1 (A1q) appeared to have a relatively low rate of genetic recombination.

The Mde BAC library was screened using AFLP 2709 as probe to identify a BAC clone (Mde37d21) containing marker 2709. Mde37d21 was then used as probe in a FISH experiment to determine its position on the polytene chromosomes. As expected, its position was near the end of A1q. However, the hybridization signal was more distal on some chromosomes than others (Figure 2A). This suggested that, among some individuals, a chromosome rearrangement was present near the end of A1q. The putative rearrangement
was observed only in females that were apparently heterozygous for the rearrangement. Polytene chromosome morphology in those individuals was also consistent with the presence of a chromosome rearrangement; the ends of the polytene A1q homologs were unpaired in putative rearrangement heterozygotes (Figure 2A), but paired in females lacking the putative rearrangement (Figure 2B).

**Contig mapping chromosome A1:** To verify the presence of a rearrangement and determine its structure, BACs from each of 74 A1 BAC contigs (AGGARWAL et al. 2009) were used as FISH probes to determine the order of the contigs on putatively rearranged and non-rearranged A1 chromosomes. These experiments revealed that near the Mde37d21 position, three contigs (#367, #1288, and #1530) had an inverted order on approximately one-quarter of the chromosomes examined (data not shown). This indicated that the putative rearrangement was a small inversion (Figure 3). BACs Mde37d21 and Hf7b16 confirmed this hypothesis by clearly defining inversion breakpoints (Figure 2C). The relative length of the inversion, hereafter called $In(A1q1)$, indicated it was only $2.1 \pm 0.2$ Mb long (1.3% of the genome). Interestingly, a second group of six contigs (#1267, #94, #252, #302, #643, and #360) also had an inverted order in approximately one-quarter of the chromosomes in several preparations (data not shown). This suggested the existence of a second, more proximal, and non-overlapping inversion (Figure 3). This inversion’s breakpoints were apparent in FISH experiments that used both BAC Hf5d5 and BAC CL1c14 as probes (Figure 2D). The relative length of this inversion, hereafter called $In(A1q2)$, indicated that it was $3.2 \pm 0.6$ Mb long (2% of the genome). We used Mde37d21
as a diagnostic FISH marker for In(A1q1) and Hf5d5 as a diagnostic FISH marker for In(A1q2) in all subsequent experiments.

**Autosome-1 inversions in Hessian fly populations:** To examine the prevalence of the inversions, we examined their distribution in nine Hessian fly populations (Table 1). These populations had been collected independently from wheat fields across the United States from 1955 to 2005 and from Israel in 2008. They were maintained in the greenhouse as large (N>1x10⁶) separate populations. Using FISH, we examined 10 individuals within the broods produced by individual females taken from these populations. In(A1q1) was observed in every population (Table 1). In(A1q2) was observed in all populations except Georgia E, Georgia vH9, Indiana D, and Israel. The inversions were never homozygous, and In(A1q2) was never present unless In(A1q1) was also in cis. Thus, three A1 variants were observed (Table 1, Figure 2). These were named A1-prime-1 (A1’1), which carried only In(A1q1); A1-prime-2 (A1’2), which carried both In(A1q1) and In(A1q2); and A1, which lacked either inversion.

A1 and A1’ chromosome distribution matched the distribution of Cm (Figure 1BC, Table 1): Females in each all-female brood (n=51) were composed of A1’[1 or 2]/A1 (Cm/-) heterozygotes and A1/A1 (-/-) homozygotes. Males in each all-male brood (n=39) were always A1/A1 (-/-). The chromosomes of both males and females in nine amphygenic broods lacked inversions. The Kansas amphygenic broods were subsequently inbred by full-sib matings for 2 consecutive generations as amphygenic populations lacking A1 inversions. The presence of In(A1q1) in the Israeli population (Figure 3EF) indicated that In(A1q1) evolved before the introduction of the Hessian fly into the new world.
Correspondence between A1 inversions and the thelygenic phenotype: To test the correspondence between \( Cm \) and the inversions further, a single male selected from an all-male A1/A1 family was mated with both a thelygenic female and an arrhenogenic female taken from an [A1/A1; A1’2/A1] all-female family (Figure S2). The F1 female offspring of the thelygenic female were then allowed to freely inter-mate with the F1 male offspring of the arrhenogenic female. The F2 adults were also permitted to freely inter-mate. FISH diagnostics were then used to karyotype the polytene chromosomes of 10 individuals within each of the broods produced by 86 individual F2 females. Again, the correspondence between \( Cm \) and A1’2 was perfect. Each all-female F3 family (n=48) contained both A1’2/A1 (\( Cm/- \)) and A1/A1 (-/-) females. Therefore, assuming that F2 males (-/-) never transmitted A1’2, thelygenic F2 females (\( Cm/- \)) always carried A1’2. Consistent with this observation, each all-male F3 family (n=38) contained only A1/A1 (-/-) males. Thus, the arrhenogenic F2 females (-/-), that produced the F3 males, clearly lacked A1’2. No recombination between \( \text{In}(A1q1) \) and \( \text{In}(A1q2) \) was observed among the 860 individuals examined.

A1 inversions in exceptional males: Chromosome non-disjunction occasionally occurs during Hessian fly oogenesis, producing ova that lack X chromosomes (A1A2OO) (STUART and HATCHETT 1991). After fertilization and chromosome elimination, the somatic cells of the zygotes produced by these ova have a male-determining karyotype (A1A2OO/A1A2X1X2). If their mother is thelygenic, these zygotes develop as “exceptional males” in female broods (Figure S3A). These exceptional males transmit their
maternally derived autosomes and their paternally derived X chromosomes to their offspring (Figure S3B). We reasoned that because exceptional males can inherit $Cm$ from a thelygenic mother, that they could be used to test the correspondence between the inversions and the thelygenic phenotype and determine if the inversions are homozygous viable.

Nine exceptional males were discovered in the brood of a single Missouri A1’2/A1 thelygenic female. To determine whether any of these had inherited A1’2 from their mother, these males were permitted to mate with multiple sisters. Each mated female was then separately caged on wheat seedlings, and the polytene karyotypes of at least 10 of her offspring were examined. Only six males successfully mated. FISH diagnostics determined that four of these transmitted A1 and two transmitted A1’2. As expected (Figure S3B), the males that transmitted A1’2 ($Cm$) produced male broods that were A1/A1’2 (-/Cm) (n=7; Figure 5A), and female broods that were segregating for A1/A1’2 (-/Cm) and A1’2/A1’2 ($Cm/Cm$) (n=6; Figure 5BC). Males that transmitted A1 (-) produced male broods that were A1/A1 (-/-) (n=6) and female broods that were segregating for A1/A1 (-/-) and A1’2/A1 ($Cm/-$) (n=4, data not shown). No recombination between the inversions on the A1’2 was observed. The discovery of A1’2 homozygous female larvae (Figure 5C) clearly indicated that the A1’2 chromosome was homozygous viable.

The remaining females in three [A1/A1; A1’2/A1] female broods and four [A1/A1’2; A1’2/A1’2] female broods were allowed to develop, mate, and reproduce on separate caged pots of wheat seedlings. The sexes of the broods produced by these females were then determined. The results confirmed the correspondence between the inversions and the thelygenic phenotype (Table 2): Both thelygenic ($Cm/-$) and arrhenogenic (-/-) females
were present in equal numbers in [A1’2/A1; A1/A1] broods, but only thelygenic females
(Cm/-; Cm/Cm) were present in [A1/A1’2; A1’2/A’2] broods.

**Recombination frequencies over the long arm of chromosome A1:** To test for
recombination suppression along the long arm of each A1 variant, five polymorphic A1q
molecular markers were genetically mapped using three mapping populations (Figure 5).
The first population consisted of the male offspring of an arrhenogenic female (-/-) lacking
A1 inversions (A1/A1; n=54). The second population consisted of the female offspring of
an A1’1/A1 thelygenic (Cm/-) female (n=50), and the third population consisted of the
female offspring of an A1’2/A1 thelygenic (Cm/-) female (n=132). To map Cm, the
females in the latter two populations were genotyped as Cm/- and -/- by sexing their
offspring. In the absence of inversions, only the two most proximal markers failed to
recombine. However, in the presence of In(A1q1), there was no recombination between
Cm and the two most distal markers, 27b3 and SSR 71. This suggested that In(A1q1)
suppresses recombination in the distal region of A1q where Cm resides. Interestingly,
when In(A1q1) was present, recombination appeared to increase between the more
proximal markers on the chromosome. When both In(A1q1) and In(A1q2) were present, all
recombination between all but the two most proximal markers (SSR 113 and 3L9b) was
completely eliminated. In(A1q2) therefore appeared to extend recombination suppression
with Cm. This was consistent with the absence of recombination we had observed between
the inversions in previous experiments.

DISCUSSION
We hypothesize that the A1 inversions are a thelygenic-determining neo-sex-chromosome. Other sex chromosomes have evolved when a dominant, sex-determining, genetic factor became the master switch in the sex determination pathway (Bull 1983; Charlesworth 1978; Charlesworth 1996; Rice 1996). An inversion then evolved near this factor to prevent the breakup of allele combinations that had sex-specific benefits. The recombinationally isolated chromosomal segment was then subject to the gene eroding force of Muller’s ratchet. This process is visible in the remnants of the genes that were once shared by the proto-sex chromosomes of humans (Hughes et al. 2005; Lahn and Page 1999), other mammals (Graves and Shetty 2001), and birds (Ellegren and Carmichael 2001; Handley et al. 2004). It has also been observed in the chromosomes of snakes (Jones and Singh 1985), plants (Liu et al. 2004b; Nicolas et al. 2004; Zluvova et al. 2005), and fungi (Lengeler et al. 2002), as well as autosome-sex chromosome translocations (Bachtrog 2005; McAllister 2003; Steinemann and Steinemann 1998), and synthetic sex chromosomes (Rice 2002).

We believe the A1’ inversions provide additional evidence of this process. We note that the A1’ chromosomes have three properties that are associated with neo-Y and neo-W chromosomes (Charlesworth 1978): 1) They carry the dominant master switch of sex determination (Cm), 2) they are normally present in a heterozygous state, and 3) they are associated with inversions that suppress genetic recombination. Genetic decay was not observed as the A1q inversions proved to be homozygous viable. However, this may be attributed to either a lack of evolutionary time, or to the frequency in which exceptional males make these chromosomes homozygous in nature.
A chronology that is consistent with the evolution of a neo-sex-chromosome is also evident in the distribution of the A1’ inversions. Before Cm arose, females would have produced offspring of both sexes. Amphygenic females have this primitive condition and lack A1 inversions. After Cm evolved, an A1 inversion would then be expected to arise to restrict recombination between Cm and other genes with sex-associated roles. Consistent with this expectation, In(A1q1) and In(A1q2) are present on the Cm-bearing chromosome. It is possible that In(A1q1) is the Cm mutation. If so, then In(A1q2) represents the first recombination suppressing inversion. If In(A1q1) is not the Cm mutation, then In(A1q2) represents an extension of recombination suppression; one that is reminiscent of the stratification that occurred along the human Y (LAHN and PAGE 1999) and the chicken W (HANDLEY et al. 2004). After the evolution of these chromosomes, we suspect that an arrhenogenic A1 probably evolved to complement its thelygenic A1’ counterparts, and together these chromosomes engendered the monogenic phenotypes that are predominant in Hessian fly populations. However, monogeny is disadvantageous when the family of an individual female becomes geographically isolated from all others. We suspect that this disadvantage has both maintained amphygenic genotypes in Hessian fly populations and promoted alleles that favor the production of exceptional males.

The previous discovery of inversions that control post-zygotic sex determination also supports our hypothesis. The X-prime (X’) chromosomes of Sciara coprophila and S. impatien, discovered in the 1930’s, carry an inversion and a maternal effect master switch, the “prime factor” (PF), that controls X chromosome elimination (GERBI 1986; METZ 1938; SÁNCHEZ and PERONDINI 1999). X-linkage obviously distinguishes PF from Cm. However, this distinction is superficial. Because the master switch acts maternally and the
maternal karyotypes are diploid for all chromosomes, X’ and A1’ are functionally
equivalent. Both chromosomes engender female development, and therefore may be
classified as maternal effect neo-Ws; we suggest the term “W-prime” (W’), where “prime”
represents the maternal effect. Unlike a neo-W, these neo-W’s establish thelygenic females
(ZW’) as the heterogametic “sex,” and arrhenogenic females (ZZ), and males (ZZ or ZO) as
the homogametic “sexes.” Thus, the genetic conflicts that act as a force in the evolution of
a sex chromosome (PARTRIDGE and HURST 1998; RICE et al. 2008; WERREN and
BEUKEBOOM 1998) are probably altered when the chromosome is a neo-W’.

The discovery of the A1’ inversions led us to question whether similar inversions might
exist in other monogenic species. Bell (1982) placed these into three catagories: cyclically
parthenogenetic taxa (cladocerans, rotifers, and cynipid wasps), ectoparasitic arthropods
(e.g. Pediculus), and a “mixed bag” of cecidomyiids, sciarids, isopods, amphipods, and
centipedes. More recently, symphypleonan springtails have been shown to belong in the
latter category (DALLAI et al. 1999), and monogeny has been more firmly established
among cyclically parthenogenic cynipids (STONE et al. 2002), certain aphids (MILLER and
AVILÉS 2000; RISPE et al. 1999), and Daphnia (Crustacea: Cladocera) (INNES and
DUNBRACK 1993). At least one cyclically parthenogenetic nematode may also be
monogenic (ALBERTSON et al. 1979). Interestingly, it is possible that the anomalous
Hessian fly chromosome cycle may have evolved in a cyclically parthenogenetic gall midge
(NICKLAS 1960) as different groups of primitive gall midges utilize this life cycle
(MATUSZEWSKI 1982; NORMARK 2003; ROSKAM 2005).

The molecular mechanisms that underlie the maternal effect of sciarid X’ chromosomes
have been thoughtfully modeled by others (DE SAINT PHALLE and SULLIVAN 1996; HAIG
1993; SÁNCHEZ and PERONDINI 1999). We will not attempt to review those models here. However, while we do not suggest that $Cm$ is necessarily homologous to the sciarid PF, we do note that the relatively small size of $In(A1q1)$ presents an excellent opportunity to identify a candidate PF and examine the veracity of those models. Finally, we note that the A1’2 inversions would make an excellent target for foreign DNA constructs. Because the paternally derived chromosomes are always eliminated from the sperm (Fig. 1A), insertions into the other autosomes and X chromosomes would be lost when males carrying the inserted construct mate only with arrhenogenic females. The A1’2 inversions would protect a foreign construct from this type of loss because they insure maternal transmission.
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### TABLE 1

**A1 variants in the broods of single Hessian fly females**

<table>
<thead>
<tr>
<th>Source Population&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number of broods (A1 karyotypes of brood)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>All-female</th>
<th>All-male</th>
<th>Amphygenic&lt;sup&gt;c&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>4 (A1/A1; A1/A1’2)</td>
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<tr>
<td></td>
<td>1 (A1/A1; A1/A1’2)</td>
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<tr>
<td></td>
<td>3 (A1/A1; A1/A1’2)</td>
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<tr>
<td></td>
<td>8 (A1/A1; A1/A1’2)</td>
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<sup>a</sup>The Country or State (year) from which the population was collected.

<sup>b</sup>A1= A1 chromosome lacking inversions; A1’1= A1 chromosome carrying In(A1q1); A1’2= A1 chromosome carrying In(A1q1) and In(A1q2).

<sup>c</sup>Amphygenic broods had greater numbers of males (Mean = 80±6%) than females.
TABLE 2

The A1 karyotypes and reproductive phenotypes of the female offspring of exceptional males

<table>
<thead>
<tr>
<th>Female brood</th>
<th>A1 karyotypes within broods</th>
<th>Reproductive phenotypes</th>
<th>Thelygenic</th>
<th>Arrhenogenic</th>
<th>Chi-square</th>
<th>Prob.*</th>
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<tr>
<td>(39x39)-21</td>
<td>A1 /A1’2; A1’2/A1’2</td>
<td>24*</td>
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<td>24.00</td>
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<td>21*</td>
<td>0</td>
<td>21.00</td>
<td>0.0001</td>
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*Probability that the number of thelygenic and arrhenogenic females were equal.
FIGURE LEGENDS

FIGURE 1.— Chromosome behavior and sex determination in the Hessian fly. (A) Syngamy (1) establishes the germ line chromosome constitution: ~32 maternally derived E chromosomes (represented as a single white chromosome), and both maternally derived (black) and paternally derived (grey) autosomes and X chromosomes. During embryogenesis, while the E chromosomes are eliminated, the paternally derived X chromosomes are either retained (2) or excluded (3) from the presumptive somatic cells. When the paternally derived X chromosomes are retained (2), a female determining karyotype is established. When they are eliminated (3), a male determining karyotype is established. Thelygenic mothers carry Cm (white arrow), which conditions all of their offspring to retain the X chromosomes. Recombination occurs during oogenesis (4). All ova contain a full complement of E chromosomes and a haploid complement of autosomes and X chromosomes. Chromosome elimination occurs during spermatogenesis (5). Sperm contain only the maternally derived autosomes and X chromosomes. (B) The segregation of Cm (white dot) on a Hessian fly autosome among monogenic families. Thelygenic females produce broods composed of equal numbers of thelygenic (Cm/-) and arrhenogenic (-/-) females (box 1). Arrhenogenic females produce males (box 2). (C) Matings between monogenic and amphygenic families. Cm (white dot) is dominant to the amphygenic-derived chromosomes (grey dot) and generates all-female offspring (box 3). Amphygenic-derived chromosomes are dominant to the arrhenogenic-derived chromosomes (no dot) and generate offspring of both sexes (box 4).
FIGURE 2.— Fluorescence in situ hybridization (FISH) revealed A1q inversions. Each panel shows a pair of DAPI stained A1 polytene chromosomes with the positions of the centromere (arrow) and the nucleolus (N) indicated. The hybridization signals associated with the BAC clones that were used as probes are labeled consistently across panels: Mde37d21, green dots; Hf5d5, red dots; CL1c14, blue dots; Hf7b16, red squares. The positions of the more distal inversion, In(A1q1) (1), and the more proximal inversion, In(A1q2) (2), are marked with white lines. (A) A distal shift of Mde37d21 hybridization was diagnostic for In(A1q1) in A1’1/A1 females. (B) Polytene chromosome lacking inversions. (C) A1’2/A1 polytene chromosome pair showing the red fluorescence of BAC Hf7b16 (red squares) at the In(A1q1) breakpoints. (D) A1’2/A1 polytene chromosome pair showing the red fluorescence of the BAC Hf5d5 (red dots) at the In(A1q2) breakpoints. (E-F) Sisters taken from the Israel population were segregating as In(A1q1) heterozygotes (E) and non-In(A1q1) homozygotes (F).

FIGURE 3.— FISH-based map of BAC-based contigs along Hessian fly chromosome A1. The chromosome is diagramed as a dark vertical line and the positions of the centromere (constriction) and nucleolus (N) are indicated. Horizontal lines indicate the relative positions of each of 74 numbered contigs. The number of BACs in each contig and the BACs used as probes to position the contig on the chromosome are indicated. BACs from the CL library are preceded by the designation “CL.” All other BACs are from the Hf library. A dash between the two clones used as probe indicates that it was possible to order
their positions on the chromosome; the first clone listed was most proximal. A comma between the BACs indicates that the orientation of the contig could not be determined. \textit{In(A1q1)} (arrow 1) and \textit{In(A1q2)} (arrow 2), were identified on the long arm of chromosome A1. The order and orientation of the contigs within the inversions was the reverse of that shown on the map.

FIGURE 4.– A1 chromosomes inherited from an exceptional male. DAPI stained polytene chromosomes show the green hybridization signal of BAC Mde37d21 (green dots) and the red hybridization signal of BAC Hf5d5 (red dots). All images are shown at the same magnification. (A) Matings between the exceptional male and an arrhenogenic female produced male offspring with A1 polytene chromosomes heterozygous for chromosome A1’2 and A1. (B-C) Matings between the same exceptional male and a thelygenic female produced female offspring that were either heterozygous A1’2/A1 (B) or homozygous A1’2 (C). The horizontal white bar represents 10 µm.

FIGURE 5.– A1 chromosome inversions influence A1q recombination. (A) The relative physical positions of five DNA markers and \textit{In(A1q1)} (arrow 1) and \textit{In(A1q2)} (arrow 2) are shown next to a giemsa stained polytene chromosome lacking the inversions. The positions of the centromere (arrowhead) and the nucleolus (N) are indicated. To the right of the chromosome are maps B, C, and D showing the relative genetic positions of the same markers and the \textit{Cm} locus (where possible). Mapping populations consisted of the
offspring of (B) an arrhenogenic female (A1/A1; n=54), (C) a thelygenic female heterozygous for In(A1q1) (A1’1/A1; n=50), and (D) a thelygenic female heterozygous for both In(A1q1) and In(A1q2) (A1’2/A1; n=132). Genetic distances (cM ± SE) between markers are indicated to the right of each map. Genetic distances that are significantly different between maps are indicted by the letters “a,” differences between maps A and B; “b,” differences between maps A and C; and “c,” differences between maps B and C (chi-square two-way test, p < 0.01).