Note

Haldane's Rule by Sexual Transformation in Caenorhabditis

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ABSTRACT

Haldane's rule in *C. briggsae* × *C. remanei* broods was caused by sexual transformation; XX and XO hybrids were female. *C. briggsae* and *C. remanei* variants that partially suppress hybrid sexual transformation were identified. Effects of variant strains were cumulative. Hence, aberrant sex determination is a reproductive isolation mechanism in Caenorhabditis.

SPECIATION occurs when two populations become reproductively isolated (Mayr 1963). Postzygotic isolation is thought to arise when geographically separate populations become fixed for mutations that, while individually neutral or advantageous, are dysgenic in combination (Bateson 1909; Dobzhansky 1937; Muller 1940, 1942). This model does not address the genetic and molecular mechanisms of dysgenesis nor is it predictive of the number of genes involved in reproductive isolation. These gaps have been filled through analyses of recurrent patterns in speciation and through studies of reproductive isolation genes.

One recurrent pattern of speciation is Haldane's rule: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex" (Haldane 1922). This rule is obeyed in mammals, flies, fish, birds, butterflies, and amphibians (Laurie 1997). This broad adherence to Haldane's rule implies that speciation in diverse taxa proceeds through similar mechanisms. Hence, Haldane's rule has received considerable attention in speciation theory (*e.g.*, Charlesworth *et al.* 1987; Wu and Davis 1993; Orr 1997; Singh and Kulathinal 2000; Turelli and Orr 2000).

In nematodes, Haldane's rule is observed in the cross of *Caenorhabditis briggsae* AF16 males to *C. remanei* EM464 females (Baird *et al.* 1992; Table 1, Figure 1). Most AF16::EM464 hybrids arrested during embryogenesis but those that did reach adulthood were female. Vulvae were present in most F_1 adults and many had multiple pseudovulvae. Vulvae typically were protruding and otherwise abnormal. In no F_1 hybrids was any evidence of male tail development observed. In the reciprocal cross, all hybrids arrested during embryogenesis (Baird *et al.* 1992).

Gonads of adult *C. briggsae::C. remanei* hybrids were abnormal and often were not useful for gender identification (Figure 1, F and G). They frequently were enlarged in size and in cell number relative to gonad primordia but exhibited no directed outgrowth or somatic differentiation (Figure 1F). In hybrids in which directed outgrowth was apparent, gonads exhibited the female/hermaphrodite morphology and usually were incompletely developed and/or degenerate (Figure 1G). In some, apparently functional spermathecae containing sperm were observed (Figure 1G). In a previous study, an exceptional hybrid containing a single arrested F_2 zygote was obtained (Baird *et al.* 1992).

The absence of males from AF16::EM464 broods resulted from sexual transformation, not male-specific lethality. This was determined using a single-worm PCR assay to detect the *C. briggsae* homolog of the X-linked *unc-18* gene (*Cb_unc-18*). Detection was expected in diplo-X but not in haplo-X hybrids. *Cb_unc-18* was detected in only one-half of the adult female AF16::EM464 hybrids tested (Table 1, Figure 2). In retrospective observations of AF16::EM464 micrographs, no correlation was detected between karyotype and adult anatomy (Figure 1, D and E).

Partial suppression of hybrid sexual transformation was observed in crosses that used the *C. briggsae* HK104 and *C. remanei* PB228 strains. Adult females and intersexes were observed among HK104::EM464 and AF16::PB228 hybrids (Table 1). Intersexes typically had a rudimentary vulva or multiple pseudovulvae and exhibited some degree of male tail specialization (Figure 3); phenotypes were similar to those of partially transformed *C. elegans* mutants (*e.g.*, Hodgkin 1987). Among HK104::EM464 hybrids, intersexes constituted less than one-half of all adults (Table 1). This departure from a male/

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TABLE 1

Sex and karyotype ratios of adult *C. briggsae::C. remanei* hybrids

Cross[a]	All adults		XX adults:	XO adults:	Hybrid
	Female:male[b]	% male	female:male[b]	female:male[b]	viability[c]
AF16 × EM464	109:0	0.00	21:0	28:0	4.4 (549)
AF16 × PB228	43:45	0.51	4:0	0:6	4.2 (721)
HK104 × EM464	25:13	0.34	6:1	4:4	3.7 (405)
HK104 × PB228	20:47	0.70	6:0	2:31	4.1 (834)

[a] All crosses, *C. briggsae* males × *C. remanei* females. Matings were conducted at 25° as described by Baird *et al.* (1992). Outcomes of crosses were the same in preliminary tests conducted at 15° (not shown). Strains were *C. briggsae* AF16 from Gujarat, India (Fodor *et al.* 1983; *C. briggsae* HK104 from Okayama, Japan (Baird 2001); *C. remanei* EM464 from Brooklyn, New York (Baird *et al.* 1994); and *C. remanei* PB228 from Englewood, Ohio (Baird 1999), available from the *Caenorhabditis* Genetics Center (http://biosci.umn.edu/CGC/CGChomepage.htm).

[b] Male count includes male and intersexual hybrids.

[c] Percentage of hybrid eggs laid that matured to adulthood. P_0's were transferred daily to fresh plates. Eggs were counted immediately following transfer of P_0's. Adults were counted as they matured. Number of eggs laid is indicated in parentheses. Note that *C. sp. v* and *C. remanei* EH of Baird *et al.* (1992) correspond to *C. remanei* EM464 and CB5161, unnamed species of Caenorhabditis, respectively (Sudhaus and Kiontke 1996).

intersex frequency of 0.50 was accounted for by the presence of some fully transformed XO females (Table 1, Figure 2). XO females were not present among AF16::PB228 hybrids and an adult intersex frequency of 0.5 was obtained (Table 1, Figure 2).

Effects of *C. briggsae* HK104 and *C. remanei* PB228 variants were cumulative; males rather than intersexes were observed among HK104::PB228 adult hybrids (Figure 3). Despite this, some XO hybrids were female. The presence of XO females was unexpected, especially considering the lack of XO females among AF16::PB228 hybrids. Another anomaly was the preponderance of HK104::PB228 males (Table 1). This male bias probably resulted from female-specific lethality as no XX males were observed among adult hybrids (Table 1).

The *C. briggsae* male to *C. remanei* female cross is the only example in which Haldane's rule is unambiguously caused by sexual transformation. This mechanism was suggested by Haldane (1922) on the basis of the apparent transformation of WZ hybrids in lepidopterans

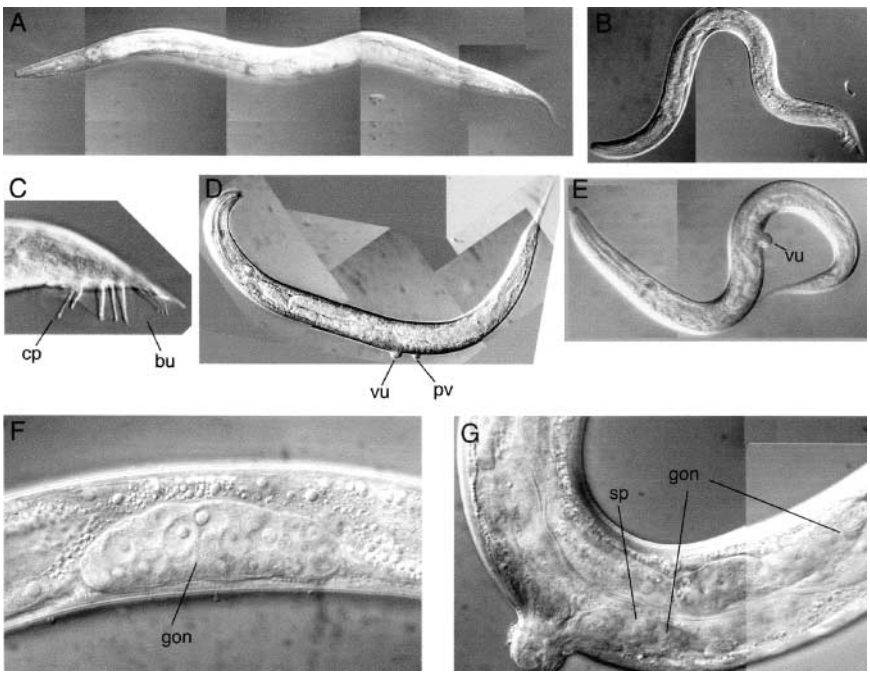

Figure 1.—Sexual transformation of *C. briggsae* AF16::*C. remanei* EM464 hybrids. (A) A *C. briggsae* hermaphrodite that is anatomically identical to *C. remanei* females. (B) A *C. briggsae* male. (C) An enlarged view of the tail of a *C. briggsae* male. Indicated are the nine bilateral pairs of male-specific caudal papillae (cp, cp1 indicated) and the copulatory bursa (bu). Not apparent at this focal plane are the male-specific spicules and sensory hook. (D) An XO hybrid exhibiting an aberrant vulva (vu) at midbody, a posterior pseudovulva (pv), and a tapered female tail. (E) An XX hybrid exhibiting an aberrant vulva (vu) at midbody and a tapered female tail. (F) Midbody of an adult hybrid showing an enlarged undifferentiated gonad (gon). (G) Midbody of an adult hybrid showing the posterior arm of a reflexed hermaphrodite gonad (gon) that contained sperm (sp) within the spermatheca. Worms were mounted on thin pads of 2% agarose for microscopic observations using differential interference contrast optics (Sulston and Horvitz 1977). Micrographic images were digitally captured using a Spot camera and software (Diagnostic Instruments, Sterling Heights, MI).

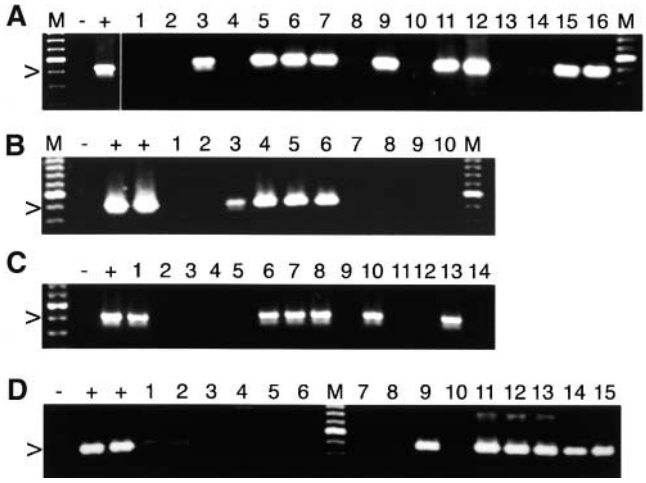

Figure 2.—Karyotypes of hybrid adults. *Cb_unc-18* was detected in single-worm PCR assays (Williams *et al.* 1992). Primers were TGCAATTATAGTAAAACTCCGTCG and TGTTCAA GTTCTCGTCAGTGATTCC. Amplification profile was 95° for 5 min followed by 30 cycles of 95° for 30 sec, 56° for 30 sec, 72° for 2 min, followed by 72° for 10 min. From these primers a 506-bp amplification product was expected. (A) AF16::EM464 female hybrids (lanes 1–16). (B) AF16::PB228 female (lanes 3–6) and male (lanes 1 and 2 and 7–10) hybrids. (C) HK104::EM464 female (lanes 6–14) and male (lanes 1–5) hybrids. (D) HK104::PB228 female (7–15) and male (1–6) hybrids. Positive controls (+), single-worm amplifications of *C. briggsae*. Negative controls (−), single-worm amplifications of *C. remanei*. Size markers (M), 100-bp ladder; position of 500-bp marker is indicated (>). The expected amplification product was obtained in 93% of all control reactions with *C. briggsae* AF16 and HK104 ($N = 41$). Amplification products never were observed in control reactions with *C. remanei* EM464 ($N = 13$). Faint amplification products were observed in one of eight control reactions with *C. remanei* strain PB228. These amplification products were not as robust as those obtained in *C. briggsae* reactions.

(Harrison 1919; Goldschmidt 1934). For *Lymantria dispar*, this interpretation has been disproved (Clarke and Ford 1980, 1982, 1983). Sexual transformation also was proposed for *Anas platyrhyncha::Cairina moschata* duck hybrids (White 1945). However, the affected WZ hybrids did not exhibit any exclusively male characters and may simply have been poorly developed females (Crew and Koller 1936). Sexual transformation does occur in *Mus poschiavinus::M. domesticus* mouse hybrids but not in the F_1 generation (Eicher *et al.* 1982). Finally, sexual transformation has been reported for *Drosophila repleta::D. neorepleta* hybrids (Sturtevant 1946). This cross is not an example of Haldane's rule as it was homogametic hybrids that were transformed and, as in mouse, transformation of *D. repleta::D. neorepleta* hybrids did not occur or was rare in the F_1 generation. In all other instances that have been investigated, the absence or rarity of the heterogametic sex from the F_1 generation has been the result of gender-biased lethality (Laurie 1997). Dosage compensation, which shares some genetic elements with sex determination, has been ruled out as a cause of sterility and inviability in *D. melanogaster::D. simulans* hybrids (Orr 1989).

In *C. elegans*, sex determination and dosage compensation are set according to the X-to-autosome ratio (Meyer 1997, 2000). This ratio is interpreted by a negative regulatory genetic pathway. Numerator elements on the X chromosome and upstream genes are required for both dosage compensation and sex determination. Downstream, this pathway bifurcates, with dosage compensation and sex determination being regulated separately. This mechanism of sex determination has been conserved, at least in part, in *C. briggsae* and *C. remanei*. Segregation of sex-linked mutant phenotypes in *C. briggsae* and *C. remanei* was consistent with the presumed XX and XO karyotypes of hermaphrodites/females and males, respectively (LaMunyon and Ward 1997; S. E. Baird, unpublished results). Moreover, *C. briggsae*, like *C. elegans*, is a self-fertile hermaphrodite in which males are rare among self-progeny (Dougherty and Nigon 1949). *C. briggsae* male frequency increases to ~50% following matings between hermaphrodites and males, consistent with segregation of nullo-X sperm from XO males (LaMunyon and Ward 1997). Finally, functional

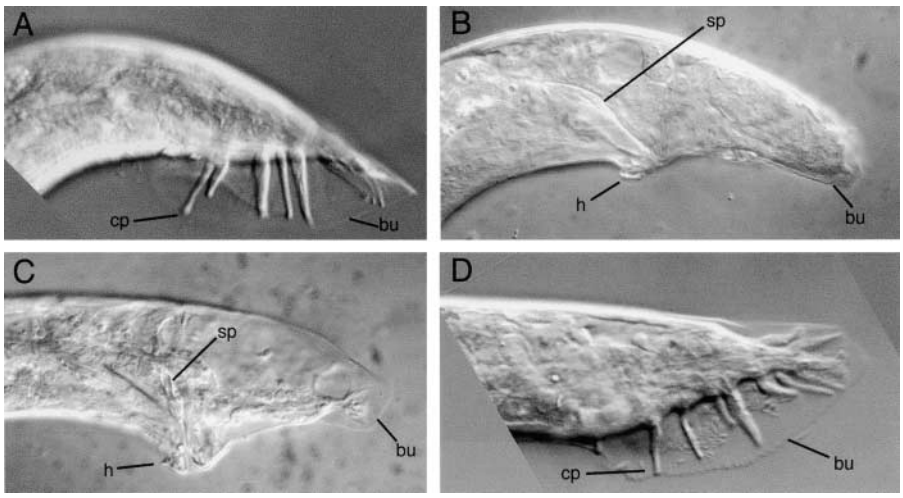

Figure 3.—Tail phenotypes of *C. briggsae::C. remanei* XO hybrids. (A) Lateral view of *C. briggsae* male. (B) Lateral view of HK104::EM464. (C) Lateral view of AF16::PB228. (D) Lateral view of HK104::PB228. Male-specific characters include the copulatory hook (h), the bursa (bu), the nine bilateral pairs of caudal papillae (cp, not all indicated), and the spicules (sp). Note also the retraction of the tail tip in male/intersexual hybrids.

conservation of several genes in the sex determination branch of the sex determination/dosage compensation pathway has been demonstrated in *C. briggsae* and *C. remanei* (De Bono and Hodgkin 1996; Kuwabara 1996; Hansen and Pilgrim 1998; Streit *et al.* 1999; Haag and Kimble 2000; E. S. Haag, S. Wang and J. Kimble, personal communication).

Dysgenic interactions among *C. briggsae* and *C. remanei* genes that affect sex determination but not dosage compensation are a likely cause of hybrid sexual transformation. These genes are among the most highly divergent in Caenorhabditis (De Bono and Hodgkin 1996; Kuwabara 1996; Hansen and Pilgrim 1998; Streit *et al.* 1999; Haag and Kimble 2000). Genes required for dosage compensation are less likely candidates because their loss-of-function phenotype is gender-specific lethality (Meyer 1997, 2000). Of particular interest is the rapid coevolution of *tra-2* and *fem-3* orthologs (E. S. Haag, S. Wang and J. Kimble, personal communication). In *C. elegans*, physical interaction of TRA-2 and FEM-3 proteins is a crucial step in the sex determination pathway (Mehra *et al.* 1999). This interaction is conserved in *C. briggsae* and *C. remanei* (E. S. Haag, S. Wang and J. Kimble, personal communication) despite the fact that the FEM-3 binding domains of TRA-2 orthologs are hypervariable (Mehra *et al.* 1999; Haag and Kimble 2000). Because of this hypervariability, TRA-2 and FEM-3 interactions are species specific (E. S. Haag, S. Wang and J. Kimble, personal communication). This type of species specificity of sex determination protein interactions is anticipated to be the basis of hybrid sexual transformation in Caenorhabditis.

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