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Evidence of Heterozygosity in Parthenogenetic Turkeys from Homograft Responses. (28409)

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Reports that all parthenogenetic turkeys have been males(1,2) and that their somatic cells carry the diploid chromosome complement(3-5) have prompted speculation as to their cytological route of origin. Beatty has discussed such routes in detail(6). It has been suggested that these turkeys arise either by suppression of an early cleavage division of the haploid egg or by suppression of one of the meiotic cell divisions(1,3,4,5). Suppression of an early cleavage division must produce a completely homozygous parthenogen(6). The progeny of such a bird mated to an unrelated female would be expected to carry at least one of each gene present in the sire's genotype. Homografts from such a sire to his progeny would be expected to survive permanently(7) as do homografts from a parthenogen to his dam(8). If on the other hand the homografts were rejected, it would be strong evidence that the parthenogen was heterozygous for at least one pair of histocompatibility alleles(7). Only suppression of one of the meiotic cell divisions or polar body recombination could account for such heterozygosity(6). The experiments described here were designed to test these alternatives.

Procedures. In 2 separate experiments, first and second set skin grafts were transplanted from parthenogenetic Beltsville Small White (BSW) turkey sires to their progeny by unrelated BSW females. In Experiment I, parthenogens P-10573, P-2087, and P-NB1 donated first set grafts to 2, 3 and 1 half-sib progeny respectively. (One original first set graft from P-10573 failed technically and was repeated when the second grafts were exchanged.) Second set grafts were transplanted to one progeny of each parthenogen 12 weeks after rejection of the first set.

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TABLE I. Fate of Skin Homografts from Parthenogenetic (P) and Normal (N) Turkey Sires to Progeny by Unrelated Females.

Exp	Donor band No.	No. of recipients	Results: First set	Results: Second set
I	P-10573	2	2 rejections, avg 11 days	1 rejection, 9 days*
	P-2087	3	3 " " 16 "	1 " 15 " †
	P-NB1	1	Take, 304 days	1 technical failure
II	P-10573	4	4 rejections, avg 14 days	4 grafts mummified
	P-2087	4	3 " " 14 "	3 " "
			1 take, 167 days	1 take, 137 days
Control	N-1507	3	3 rejections, avg 9 days	Not done

* No second set to one of the first set recipients.
† 2 recipients died before second set grafts could be made.

In Experiment II, P-10573 and P-2087 donated grafts to 2 male and 2 female full-sib progeny each. Second set grafts were transplanted to each of these recipients approximately 2 weeks after rejection of the first set.

Control first set grafts were transplanted from a normal BSW sire (N-1507) to 3 progeny.

All grafts were approximately 25 mm diameter discs of full thickness wattle or body skin, trimmed of subcutaneous connective tissue and fat, and sutured into prepared sites on the saddle of the recipients. The surgical procedures have been published in more detail elsewhere(8). Daily observations began at 4-5 days postgrafting and continued until the incompatible grafts were sloughed. However, they were considered rejected when they clearly were undergoing general necrosis. Compatible grafts are still under observation.

Results. Exp. I. The 2 first set grafts from P-10573 and the 3 from P-2087 were rejected in an average of 11 and 16 days respectively (Table I). One second set graft from each was rejected in 9 and 15 days respectively. The first set graft of P-NB1 is surviving 304 days postgrafting. The second set graft from P-NB1 failed technically and has not been repeated. Two progeny of P-2087 died before second set grafts could be transplanted to them.

Exp. II. Seven first set grafts were rejected in an average of 14 days. Seven second set grafts to the same birds underwent avascular necrosis and mummification typically associated with second set responses in other species(9,10). First and second set grafts to the eighth recipient, a daughter of P-2087, are still surviving at 167 and 137 days postgrafting respectively.

Control grafts from the normal male (N-1507) to 3 of his progeny were rejected in an average of 9 days.

Discussion. The second set grafts of both experiments bear further comment. In Exp. I more than 12 weeks intervened between rejection of the first set grafts and transplantation of the second set. In this time the high level of immunity which prevails immediately following a homograft reaction apparently had subsided so that the second set grafts became vascularized and enjoyed survival times nearly equivalent to first set grafts. In contrast, the second set grafts of Exp. II were transplanted within 2 weeks of rejection of the first set—the time at which one would expect highest immunity. Avascular necrosis and mummification as observed in these second set grafts has been described previously in second set grafts to mice(9) and rabbits(10), and is a typical second set response.

Two aspects of these results indicate that the parthenogenetic turkey can be heterozygous for one or more pairs of segregating histocompatibility alleles: (1) The second set responses of the progeny, particularly in Exp. II, are taken as evidence that the grafts from their parthenogenetic sires were rejected by an actively acquired homograft immunity. Thus the parthenogens' tissues must have contained antigens foreign to their progeny. (2) The fact that one graft from P-2087 to one of his progeny is surviving

although similar grafts to 6 others of his progeny were rejected is interpreted as an indication of segregation having occurred for at least one heterozygous antigenic locus.

This evidence of heterozygosity in the parthenogens has a most important bearing on the question of the cytological route by which such diploid male parthenogenetic turkeys might arise. These routes are: 1) Suppression of meiosis I, or recombination of the diploid first polar body and the nucleus of the diploid secondary oöcyte to form a tetraploid secondary oöcyte followed by a reductional division at meiosis II; 2) suppression of meiosis II, or recombination of the haploid second polar body with the haploid oötid nucleus; or 3) suppression of an early cleavage division of the mature haploid ovum.

The second route would seem to be the simplest and most likely of the 3 alternatives. Preceded by normal meiotic reduction and crossing over, it would produce the autosomal diploid equivalent of a fertilized egg containing chromosomes heterozygous within crossover regions for any loci at which the dam was also heterozygous. The sex chromosome constitution of the resulting diploid cell would be either XX (male) or OO (?). If the YY constitution is lethal in *Drosophila* (9), it is reasonable to assume the OO would be lethal in birds.

The first route, suppression of meiosis I seems unlikely. If the second meiotic division was reductional (it is normally equational for whole chromosomes) and the OO sex chromosome constitution was lethal it could produce heterozygous male parthenogens. However, if meiosis II was normally equational following suppression of meiosis I only female (XO) parthenogens would result. The latter would be contrary to prior observations(1,2).

On the basis of these results suppression of an early cleavage division is considered unlikely as the route of restoration of the diploid chromosome complement since it could only produce completely homozygous parthenogens(6).

Summary. Homografts of wattle and normal body skin from parthenogenetic Beltsville Small White turkey males to some of their progeny by unrelated females are rejected by a typical homograft reaction. The second set response to repeat grafts in the same combinations indicates that rejection of both sets was due to an active immunity acquired in response to the first set grafts. Rejection of some of the parthenogenetic sires' skin by their progeny offers strong evidence that the parthenogens are heterozygous for at least one pair of histocompatibility alleles. The importance of this finding is discussed in reference to 3 alternative cytological routes by which diploid male parthenogenetic turkeys might arise. The results would seem to eliminate suppression of an early cleavage division as a possible route since only homozygotes could thus be produced. Although suppression of meiosis II cannot be assumed *a priori* to be the route of origin it appears more likely than suppression of meiosis I.

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