We investigated the pattern of inheritance of maternal meiotic errors responsible for a high frequency of triploid progeny in a selected line of chickens. For the genetic analysis, F₁ and backcross populations were produced from crosses between normal diploid individuals of the triploidy line and a control line. Triploid embryos were produced by 35% and 67% of reciprocal F₁ females and by 24% and 67% of reciprocal backcross females. These results exclude autosomal recessive and sex-linked recessive or sex-linked dominant inheritance. A single autosomal dominant gene is also not likely to be responsible. However, the results are consistent with the determination of triploidy by a single autosomal gene with no dominance, and an even better fit is obtained by two loci, an autosomal gene with no dominance and a sex-linked gene. The results cannot exclude a multifactorial mode of inheritance, but the rapid response to selection for triploidy and consistent expression of the meiotic errors in different genotypes suggest that meiotic mutations at one or two loci are the most plausible genetic basis for the trait.

Spontaneous triploidy is occasionally observed in lower vertebrates, and in the chicken (Gallus domesticus) live triploids have been reported with some frequency (Bitgood and Shoffner 1990). Triploidy in the chicken is predominantly maternally derived, arising from diploid ova produced by chromosomal nondisjunction during oogenesis (Bitgood and Shoffner 1990). Errors at both meiotic divisions of oogenesis have been observed, with the majority occurring at meiosis II (Fechheimer 1981; Thorne et al. 1991a). It has been noted that the genotype of the dam is an important factor in the occurrence of triploidy in the fowl (Bloom 1972; Fechheimer and Jaap 1978; Thorne et al. 1991a).

We investigated the pattern of inheritance of triploidy in chickens from a selected line that produces a high frequency of triploid embryos (15-20%) and hatched triploid chickens (8-14%) (Thorne et al. 1991b). Cytological evidence has shown that the triploidy is maternal in origin (Thorne 1991; Thorne and Sheldon 1991). Females of the triploidy line produce a high frequency of diploid ova from errors at meiosis I (ZW diploid ova) and II (WW and ZZ diploid ova) of oogenesis. The consistent observation of a 2 ZZW:1 ZWW:1 ZZ sex ratio among triploid embryos from females of different generations of the line indicates that the meiotic errors occur with equal frequency at meiosis I and II. Females of the line also produce a low frequency of tetraploid ova (0.5%), as a result of failure of both meiotic divisions, and these give rise to pentaploid embryos after fertilization (Thorne and Sheldon 1991). No evidence has been found in meiotic chromosomes or in mitotic chromosomes of early embryos to show that males of the triploidy line are producing diploid spermatozoa (Thorne 1991), yet they are able to transmit the trait to their female progeny. Initial studies of the pattern of inheritance of triploidy suggested that the meiotic errors might be due to a single autosomal recessive gene (Thorne et al. 1980). We investigated this hypothesis by forming F₁ and backcross populations from reciprocal crosses between normal diploid individuals of the triploidy line and a control line known not to produce triploids. At maturity F₁ and backcross females were examined for their ability to produce triploid embryos, and the results of the cytogenetic analyses were tested against the above hypothesis.

Materials and Methods

Triploidy Line

The triploidy line (T) is a selected subline of a Synthetic layer strain that originated from a closed White Leghorn × Australorp
crossbred flock (Thorne and Sheldon 1991). For many generations, intersex ZZW triploid embryos had been observed at a frequency of 0.1–0.5% among females of the parent line. The detection of six closely related intersex triploids at a frequency of 1.2% among females of the parent line (Thorne et al. 1980) suggested the possibility of a genetic basis for the condition and initiated selection for triploidy. The first generation of the triploidy line was produced from close matings among normal diploid relatives of triploids identified by Thorne et al. (1980), and also the second generation from close matings among selected parents, but thereafter the line has been reproduced avoiding close matings.

Up to generation 5, females were selected as breeders if they produced one or more triploid embryos out of 20 embryos karyotyped at day 3 of incubation (Thorne et al. 1991b). Male breeders were selected from families where a high proportion of females had produced triploids. The response to selection for triploidy was rapid. The proportion of females producing triploid embryos was 82% by generation 5, and the incidence of triploid embryos from those females was 21% (Thorne et al. 1991b). After generation 5, female and male breeders were selected from families producing the most triploid chickens. In subsequent generations up to 95% of the females hatched triploids, but it is likely that all females were triploid producers, given the expected embryonic mortality among triploid genotypes (Thorne 1991; Thorne et al. 1991b). In each generation some females produced a high frequency of hatched triploids (40–50% of hatched chickens). Maternal age was noted not to be a factor in the production of triploids.

**Control Line**

The control line (C) is a White Leghorn strain in which triploid intersex chickens have never been observed. Cytogenetic analysis of 670 early embryos from line C revealed an extremely low incidence of chromosome aberrations (0.15%), indicating that the line was not prone to errors during meiosis and fertilization. In particular the line C females showed no tendency to produce triploid embryos (Thorne 1991). Line C was therefore assumed to be unlikely to have the genotype responsible for triploidy.

**F, and Backcross Populations**

An F, referred to as the CT flock, was formed from crosses between five line C males and 20 line T females (of generation 8). The reciprocal F, TC flock was formed from crosses between five line T males (generation 8) and 20 line C females. Two backcross populations, referred to as the CTT and CTC flocks, respectively, were formed by crossing 10 F, CT males to 20 line T females (generation 8) and to 20 line C females. It was not possible to form reciprocal backcrosses using F, TC males or backcrosses using CT or TC females due to time limits of the project.

In all cases, triploidy among triploid genotypes (Table 1) was due to two females with triploid embryos. The frequency of triploidy was due to two females with triploid embryos. Table 1 shows the distribution of the number of triploid embryos per female among 20 analyzed embryos is shown in Table 1. Triploid embryos were produced by 15% of F, CT females, 16.4% of F, TC females, 8.9% for TC, 13.9% for CTT, and 9.0% for CTC. In the CT and CTT flocks, the high frequency of triploidy was due to two females that produced 12 and 10 triploids, respectively, out of 20 analyzed embryos.

The sex chromosome ratio among 21 sexed triploid embryos from CT females was 13 ZZW:6 ZWW:7 ZZZ, and in 23 sexed triploid embryos from TC females it was 11 ZZW:9 ZWW:3 ZZZ. In 39 triploid embryos from CTT females the sex chromosome ratio was 21 ZZW:12 ZWW:6 ZZZ. In each of these cases the observed ratio was not significantly different from a 2:1:1 ratio which is expected with equal probability of failure at meiosis I and II of oogenesis (x² = 1.83, 3.24, and 2.22, respectively, for CT, TC, and CTT, df = 2, P > .1).

In nine triploid embryos produced by five CTC females, the observed sex chromosome ratio was 0 ZZW:6 ZWW:3 ZZZ, which was significantly different from the 2:1:1 ratio (x² = 10.3, df = 2, P < .01). The low number of triploid embryos in this case was consistent with an origin from errors at meiosis II where a ratio of 0 ZZW:1 ZZW:1 ZZZ is expected (x² = 1.1, df = 2, P > .5).

**Cytogenetic Analysis of Embryos of F, and Backcross Females**

Chromosomes of 20 embryos per hen were analyzed at day 4 of incubation from 20 CT females and from 21 females in each of the TC, CTT, and CTC flocks. Unrelated line C males were used as the sires to obtain fertile eggs. Pedigreed eggs were set in batches and incubated for 96 h. Chromosome preparations were made using the techniques of Bloom et al. (1972) and Shoffner et al. (1967). For each embryo, five metaphase cells were counted and sexed at 100x magnification. Chromosome aberrations were detected, 20–30 metaphase cells were counted.

**Results**

The cytogenetic analysis of 1660 embryos is presented in Table 1. Triploid embryos were produced by 35.0% of CT females and 66.7% of TC females, and by 66.7% of CTT and 23.8% of CTC females. In both the F1 and backcrosses, these proportions were significantly different (x² = 4.11 and 7.78, respectively, df = 1, P < .05).

The frequency of triploidy (Table 1) was 5.8% in CT, 6.0% in TC, 9.3% in CTT, and 2.1% in CTC. Among triploid-producing females only, the frequency was 16.4% for CT, 8.9% for TC, 13.9% for CTT, and 9.0% for CTC. In the CT and CTT flocks, the high frequency of triploidy was due to two females that produced 12 and 10 triploids, respectively, out of 20 analyzed embryos.

The sex chromosome ratio among 21 sexed triploid embryos from CT females was 15 ZZW:6 ZWW:7 ZZZ, and in 23 sexed triploid embryos from TC females it was 11 ZZW:9 ZWW:3 ZZZ. In 39 triploid embryos from CTT females the sex chromosome ratio was 21 ZZW:12 ZWW:6 ZZZ. In each of these cases the observed ratio was not significantly different from a 2:1:1 ratio which is expected with equal probability of failure at meiosis I and II of oogenesis (x² = 1.83, 3.24, and 2.22, respectively, for CT, TC, and CTT, df = 2, P > .1).

In nine triploid embryos produced by five CTC females, the observed sex chromosome ratio was 0 ZZW:6 ZWW:3 ZZZ, which was significantly different from the 2:1:1 ratio (x² = 10.3, df = 2, P < .01). The low number of triploid embryos in this case was consistent with an origin from errors at meiosis II where a ratio of 0 ZZW:1 ZZW:1 ZZZ is expected (x² = 1.1, df = 2, P > .5).

Other chromosomal abnormalities (Table 1) were observed at low frequencies (1.0% CT, 1.4% TC, 2.6% CTT, and 2.1% CTC). Included in these are 10 haploid embryos (each with a Z sex chromosome) and 3 pentaploids (each ZZZWW) of which all, except one haploid, were produced by females with triploid embryos.

A summary of the distribution of the number of triploid embryos per female among 20 analyzed embryos is shown in Table 2 for the triploidy line, F, and backcrosses. The average number of triploid embryos per female was 2.79 in the triploidy line and 0.43–1.86 in females of F1 and backcross populations.
Discussion

The most significant result of the cytogenetic analysis of early embryos of F₁ females was the occurrence of triploid embryos in 35% of CT females and in 67% of TC females. The results indicate that a single autosomal recessive gene is not likely to be responsible for the triploidy (Table 3) because under this hypothesis it is expected that none of the heterozygous F₁ CT or TC females (genotype designated as Aa for each F₁) would have produced triploid embryos. Under this model, the backcrosses (Table 4), 50% of CTT females (aa:Aa) and none of the CTC females (AA:aa) were expected to produce triploid embryos. This hypothesis was supported in the CTT backcross, but it was clearly rejected in both of the reciprocal F₁ flocks, as it was rejected as a likely mode of inheritance. The F₁ and backcross results were therefore tested against other modes of inheritance (hypotheses 2–5), presented in Tables 3 and 4.

An autosomal dominant mode of inheritance was examined in hypothesis 2. This model assumes that all of the selected line T parents were homozygous (AA). If this is the case, then the model is supported by the F₁ (Table 3) and backcross results (Table 4) because all of the heterozygous F₁ females (Aa), all CTT females (AA:aa), and 50% of CTC females (aa:Aa) should have produced triploid embryos. If, however, some of the line T parents were heterozygous Aa, then a proportion of the F₁ CT or TC females would have been homozygous aa and would not have been triploid producers. Careful examination of pedigree data supports the assumption that the line T parents were likely to have been homozygous AA. Moreover, if the gene responsible was an autosomal dominant, then a much higher frequency than the observed 0.1–0.5% of intersex triploid birds would have been expected in the parent line in successive generations prior to selection. Hypothesis 2 was thus regarded as an unlikely mode of inheritance.

Hypothesis 3 examined the possibility that a sex-linked gene might be responsible for triploidy. In this case the sex-linked gene is designated as dominant, and all of the TC females that inherited their Z chromosome from the triploid line (designated allele B) would have been expected to produce triploid embryos, compared to none of the CT females, whose Z chromosome came from line C (allele b). However, the results in both the CT and TC females do not support this hypothesis (Table 3). In the backcrosses, under hypothesis 3 (Table 4), 50% of both CTT (B:b) and CTC females (B:b) should have produced triploid embryos. Although this hypothesis was supported in the CTT backcross, it was not supported in the CTC backcross, nor in either F₁, and was rejected. The conclusions would be exactly the same for a recessive sex-linked gene.

Hypothesis 4 examined a mode of inheritance involving a single autosomal gene with no dominance. Under this model the alleles have an additive effect. All the homozygous line T females (A'A') would produce triploids, while homozygous line C females (A'A') would not have any expression, and an intermediate response of around 50% would be expected in the heterozygous F₁ females (A'A). This hypothesis was supported by the F₁ results because the observed proportion of CT females producing triploid embryos (35%) was not significantly different from 50%, nor was the observed proportion of TC females (67%) (Table 3). In the backcrosses, under hypothesis 4 (Table 4), an average of 75% of CTT females (A'A':A'A') and 25% of CTC females (A'A':A'A') would be expected to produce triploid embryos. As with the F₁ results, the observed values in both backcrosses agreed closely with the expected values. The F₁ results also suggested the possibility of a sex-linked effect influencing ex-

### Table 2. Distribution of triploid embryos per female in the triploidy line, F₁, and backcrosses

<table>
<thead>
<tr>
<th>Flock</th>
<th>Average # 3W per female</th>
<th>Number of 3W per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>2.79</td>
<td>0 1 2 3 &gt;4</td>
</tr>
<tr>
<td>CT</td>
<td>1.15</td>
<td>13 1 5 0 9</td>
</tr>
<tr>
<td>TC</td>
<td>1.19</td>
<td>7 6 6 1 1</td>
</tr>
<tr>
<td>CTT</td>
<td>0.43</td>
<td>7 4 4 4 2</td>
</tr>
<tr>
<td>CTC</td>
<td>0.43</td>
<td>16 2 2 1 0</td>
</tr>
</tbody>
</table>

*Total 3W females tested (from Table 1).  
**Triploidy line data from Thorne (1991) (generation 8 females).

### Table 3. Hypotheses tested for inheritance of ability to produce triploid embryos in F₁ females

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>F₁</th>
<th>Expected % with 3W</th>
<th>Females with 3W</th>
<th>Females without 3W</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CT</td>
<td>0</td>
<td>7 (0.0)</td>
<td>13 (20.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>CT</td>
<td>0</td>
<td>14 (0.0)</td>
<td>7 (21.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>CT</td>
<td>100</td>
<td>7 (20.0)</td>
<td>13 (0.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>CT</td>
<td>100</td>
<td>14 (21.0)</td>
<td>7 (0.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>CT</td>
<td>100</td>
<td>14 (21.0)</td>
<td>7 (0.0)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* 1 = autosomal recessive; 2 = autosomal dominant; 3 = sex-linked; 4 = autosomal, no dominance; 5 = two loci.

### Table 4. Hypotheses tested for inheritance of ability to produce triploid embryos in backcross females

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Backcross</th>
<th>Expected % with 3W</th>
<th>Females with 3W</th>
<th>Females without 3W</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CTT</td>
<td>50</td>
<td>14 (10.5)</td>
<td>7 (10.5)</td>
<td>2.56</td>
<td>.10</td>
</tr>
<tr>
<td>2</td>
<td>CTT</td>
<td>100</td>
<td>14 (21.0)</td>
<td>7 (0.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>CTT</td>
<td>50</td>
<td>5 (10.5)</td>
<td>16 (10.5)</td>
<td>0.06</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>4</td>
<td>CTT</td>
<td>50</td>
<td>14 (10.5)</td>
<td>7 (10.5)</td>
<td>2.38</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>5</td>
<td>CTT</td>
<td>75</td>
<td>14 (15.8)</td>
<td>7 (5.2)</td>
<td>0.66</td>
<td>&gt;.30</td>
</tr>
<tr>
<td>6</td>
<td>CTT</td>
<td>25</td>
<td>5 (5.2)</td>
<td>16 (15.5)</td>
<td>5.81</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>7</td>
<td>CTT</td>
<td>67</td>
<td>14 (10.5)</td>
<td>7 (10.5)</td>
<td>0.06</td>
<td>&gt;.95</td>
</tr>
<tr>
<td>8</td>
<td>CTT</td>
<td>33</td>
<td>5 (7.0)</td>
<td>16 (14.0)</td>
<td>1.05</td>
<td>&gt;.30</td>
</tr>
</tbody>
</table>

* 1 = autosomal recessive; 2 = autosomal dominant; 3 = sex-linked; 4 = autosomal, no dominance; 5 = two loci, autosomal, no dominance, and sex-linked.

* Figures in brackets are expected values.

Yates’s correction for continuity used in chi-square tests.
pression of the trait, because the observed expression of triploidy in TC females (67%) was significantly higher than in CT females (33%). Consequently, hypothesis 5 was proposed, suggesting two loci were responsible for the triploidy; one being an autosomal gene with no dominance and the other being a sex-linked gene. The simplest assumption with this model is that both the A' autosomal allele and the B sex-linked allele have equal and additive effects on the expression of triploidy. In this case then, the proportion of F₁ females producing triploids is expected to be 67% for TC females (A'A'B) and 33% for CT females (A'A'b). It is evident that the observed F₁ results are in close agreement with this hypothesis (Table 3). Hypothesis 5 also gave a close fit to the backcross results (Table 4). The average expected expression of triploidy was 67% for CTT females (A'A'B:AA'bA':A':AA'B:AA'B), and 33% for CTC females (A'A'B:A':AA'B:AA'B:A':AA'B). The observed values agreed closely with the expected values. From the results, hypothesis 5 appeared to be a more probable mode of inheritance than hypothesis 4.

In addition to considering the hypotheses discussed above, we also considered the possibility that the triploidy is multifactorial, that is, determined by a number of genetic and environmental factors. If the trait is multifactorial, then the same level of expression of triploidy would be expected in F₁ and backcross females as that expected under hypothesis 4 (autosomal gene, no dominance). The frequency distribution of the number of triploid embryos per female in each population (Table 2) was consequently investigated by probit analysis (Falconer 1989; Finney 1962) to determine if this could provide a method for distinguishing between a gene of major effect influencing the triploidy and a multifactorial model. It became apparent, though, that there was insufficient data to enable any useful inferences to be drawn.

It is evident from the studies of melotic mutants in a large number of organisms that all of the major events of meiosis are genetically controlled (Baker et al. 1976; Carpenter 1983; Golubovskaya 1979, 1989). Each of the main steps such as chromosome pairing, crossing over, spindle formation, chromosome movement to the poles, and so on are under the control of separate genes. Most melotic mutants have been observed to follow Mendelian patterns of inheritance, with a large number being controlled by single recessive genes (Curtis and Doyle 1991; Golubovskaya 1979). The melotic mutation(s) in the triploidy line is speculated to affect structures involved with chromosome disjunction such as the spindle. Melotic mutants that directly affect chromosome disjunction without interfering with pairing or crossing over have been described in plants, *Drosophila*, yeast, and *Neurospora* (Baker et al. 1976; Carpenter 1983; Golubovskaya 1979, 1989). The effects of the mutant genes vary, but they either produce spindle abnormalities or impair disjunction mechanisms resulting in a high frequency of aneuploid or diploid gametes.

**Conclusions**

We have presented the genetic analysis of a melotic mutant(s) in a higher vertebrate. The results have clearly indicated that a single autosomal recessive gene is not responsible for the triploidy, and neither is a sex-linked recessive or sex-linked dominant gene. A single autosomal dominant gene could not be conclusively rejected by the results, but it is regarded as an unlikely mode of inheritance. Evidence was provided that the triploidy might be explained by a one-locus model involving an autosomal gene with additive alleles or more plausibly by a two-locus model involving the former and a sex-linked gene. We also considered the possibility that triploidy is a multifactorial threshold trait, but it was not possible to differentiate between this model and the single-locus model involving additive alleles. We conclude, however, from the rapid response to selection for triploidy, the consistent expression of the meiotic errors in different genotypes, and evidence from the literature that melotic mutations at one or two loci are more likely to be responsible for the trait than a multifactorial model.

**References**


Received April 6, 1996

Accepted November 29, 1996

Corresponding Editor: Stephen E. Bloom