Table 1. Number of survivors in flocks of chickens between brooding and sexual maturity.

Group	Square meters per survivor	Number of survivors				
		Week 5	Week 6	Week 7	Week 8	Week 9
			Series 1			
A1	4.2	12	12	12	12	
B1	2.8	12	11	10	10	
C1	1.4	12	11	8	6	
			Series 2			
A2	4.2	12	12	11	11	11
B2	2.8	12	11	10	10	10
C2	1.4	12	10	8	7	7
D2	0.31	12	12	12	5	2

cubation, "survivorship" was examined in small flocks that were equal in initial size but occupied different areas of floor space. If some factor arising out of or associated with "experience with density" should facilitate survival, then the correlation between initial and resultant densities would be positive, and the relationship between these variables would monotonically increase. (Such a relationship, however, must have a lower and should have an upper limit.) On the other hand, if the numbers of survivors were unrelated to the chick's "experience with density," then the correlation between initial and resultant densities, under the present conditions, would be of zero order, that is, all treatment groups would reduce to about the same level.

In series 1, 36 incubated, New Hampshire–White Leghorn crossed chicks were taken from heated brooders ($N = 12$ per brooder) and randomly allocated to three flocks. The initial amount of space allotted each bird was 4.2 m^2 for group A, 2.8 m^2 for group B1, and 1.4 m^2 for group C1. Series 2 was a replicate with an additional flock ($N = 12$) that had an initial area of 0.31 m^2 per bird. In each flock, food (a medium energy commercial ration) and water were available as desired. Flocks were maintained indoors under artificial heat and light (14 hr/day).

Table 1 shows that series 1 and 2 are almost identical with respect to replicates and this was confirmed by statistical testing. The number of survivors between density levels was significantly different on the chi-square test ($p < .05$) except for those birds maintained in areas between 4.2 m^2 and 2.8 m^2 when the difference approached significance ($p = .15$).

In series 1 recordings were discontinued during week 9 since dominance fighting was observed in all treatment conditions, a result consistent with earlier studies (*5*). The number of survivors during week 9 for series 2 is given, and, although dominance encounters (but no mortalities) were then observed in groups A2, B2, and C2, no such behavior was observed in group D2. Since sexual maturity and dominance fighting coincide, it seems likely that the former may have been delayed under very high density. Similar findings (*6*) have been reported with mice.

Records of wounds and scars indicated that physical assault as a cause of death was confined to one flock only, C1. In this group all carcasses had been extensively cannibalized about the tail and saddle. This commenced in week 6 with members of C1 pecking each other's tail quills; should hemorrhage ensue the member was pecked until dead. When the resultant density was reached, however, no member died after such assaults although several hemorrhages subsequently occurred.

Within the limits of age, strain, and densities under study, a strong consistent relationship ($r = 0.999$, $p < .001$) existed between initial and resultant density. Also, within the limits specified, the function relating the variables is: Resultant density = 0.64 initial density + 1.77.

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19 September 1966

Respiratory Control: Loss in Mitochondria from Diseased Plants

Abstract. *Mitochondria from healthy oat seedlings oxidized succinate with good respiratory control and high ratios of adenosine diphosphate to oxygen. After treatment with victorin, the pathotoxin responsible for symptoms of Victoria blight of oats, susceptible seedlings yielded mitochondria with little respiratory control and lower ratios of adenosine diphosphate to oxygen. No such effects were obtained with victorin-treated resistant seedlings or when victorin was added directly to mitochondria from healthy susceptible or resistant plants. These data indicate that victorin-induced disease results in a reduction in efficiency of the energy-generating system of isolated mitochondria.*

Pathological increases in respiration are characteristic of diseased plant tissue (*1*). Attempts to determine the extent to which pathological respiration is coupled to synthesis of high-energy phosphate bonds have yielded conflicting results. In two investigations, phosphorylative-oxidative (P/O) ratios were lower in mitochondrial preparations from diseased plants than in those from healthy ones (*2, 3*); in another, no such change in P/O ratios was found during development of disease (*4*). The conflict in these results may be apparent rather than real because the procedures used, particularly the extraction of mitochondria in strong buffers, have resulted in loss of respiratory control with both animal and plant preparations (*5*). Respiratory control (RC) ratios (rate of substrate oxidation in the presence of a suitable phosphate acceptor divided by rate in the absence of such an acceptor) are considered to be better indicators of the intactness of mitochondria than P/O ratios (*5*). Mitochondria from potato tuber (*Solanum tuberosum* L.) slices inoculated with the fungus *Ceratocystis fimbriata* and incubated for 2 to 3 days had lower RC

and adenosine diphosphate to oxygen ratios (ADP/O; equivalent to P/O) than preparations from intact tubers (*6*); results with uninoculated tuber slices incubated 2 to 3 days were similar to those with inoculated ones. Furthermore, *C. fimbriata* is not a pathogen of potato tubers under natural conditions, and, therefore, these results have little bearing on the question of whether pathological respiration is efficiently geared to the synthesis of high-energy phosphate bonds. To explore this question we have studied mitochondria from diseased and healthy oat plants (*Avena sativa* L.).

We used oat seedlings treated with victorin, the pathotoxin (*7*) produced by the fungus *Helminthosporium victoriae* Meehan and Murphy. Disease was induced in susceptible seedlings (variety Victorgrain 48-93) by allowing cut stems of 8- to 9-day-old plants to take up a solution containing 20 units of victorin per milliliter for 2 hours or one with 10 units of victorin per milliliter for 4 hours. These treatments were selected because they had been shown to result in maximum increases in pathological respiration (*8*). Resistant plants (variety Camellia or C.I. 7418) were also treated with victorin. Susceptible and resistant plants exposed to solutions of deactivated victorin served as controls. Methods for production of victorin and for deactivation of victorin solutions were those previously described (*8*). All plants were grown in the dark at 25° to 27°C in large petri dishes lined with paper towels kept moist with a 1-m*M* solution of $CaCl_2$.

Mitochondria were extracted, washed, and resuspended by the methods of Wiskich and Bonner (*9*) as modified by Bonner (*5*), except that sucrose was used in most cases in place of mannitol. Oxygen uptake was followed polarographically with a vibrating platinum microelectrode. For each determination, 0.2 ml of the final mitochondrial suspension (equivalent to mitochondria from 8 g of tissue) was used in a total volume of 2 ml in the reaction vessel. This procedure was adopted after tests showed no significant differences in mitochondrial nitrogen among preparations from equal fresh weights of victorin-treated or control tissues. The ADP/O ratios were calculated by the method of Chance and Williams (*10*).

Mitochondria from victorin-treated susceptible plants showed a marked loss of respiratory control. Electrode traces from a typical experiment (Fig. 1) show that with mitochondria from control plants rates of oxygen uptake exhibited the transitions from an ADP-limited (state 3) to an ADP-stimulated rate (state 4) expected with a well-coupled system (*5, 6*). With mitochondria from victorin-treated susceptible plants, stimulations with ADP were much smaller, and the time required for a return to state 3 was much longer.

Table 1. Effect of victorin-induced disease on respiratory control (RC) and ADP/O ratios of oat mitochondria. Results are means and their standard deviations obtained in eight tests under conditions given in Fig. 1.

Source of mitochondria	Ratios	
	RC	ADP/O
Control plants		
Resistant	2.4 ± 0.20	1.2 ± 0.17
Susceptible	2.3 ± .19	1.2 ± .25
Victorin-treated plants		
Resistant	2.4 ± .32	1.3 ± .15
Susceptible	1.3 ± .26	0.6 ± .28

Results with 2- and 4-hour treatments with victorin did not differ significantly, hence the data were pooled and are summarized in Table 1. The RC and ADP/O ratios obtained with mitochondria from victorin-treated susceptible plants were significantly ($p < 0.01$) lower than those from control or victorin-treated resistant plants. None of the other differences in data in Table 1 were significant. Addition of victorin (final concentration, 20 units per milliliter) to mitochondria from untreated susceptible plants in the reaction vessel or incubation of such mitochondria with the same concentration of victorin for 2 hours at 2°C had no effect on RC or ADP/O ratios. These results extend and confirm those obtained with manometric techniques which indicated that victorin had no direct effect on mitochondria (*3, 11*) but that victorin treatment of susceptible plants resulted in a significant decrease in P/O ratios (*3*). They provide further evidence that victorin affects the respiratory centers indirectly and causes a marked reduction in efficiency of the energy-generating system of isolated mitochondria. We emphasize the fact that present data do not justify the conclusion that mitochondria in intact diseased tissues are similarly affected.

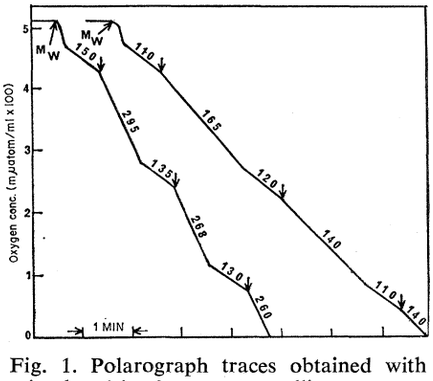

Fig. 1. Polarograph traces obtained with mitochondria from oat seedlings susceptible to Victoria blight. Lower trace represents results with control (healthy) plants and upper trace with victorin-treated (diseased) plants. Reaction vessels contained 0.3*M* sucrose, 0.02*M* succinate, 0.01*M* tris buffer, 0.1 m*M* ethylene diaminetetraacetate, 0.01*M* potassium phosphate, and 3 m*M* magnesium chloride adjusted to *p*H 7.2. Mitochondria (M_w) containing 390 μg of nitrogen were added after 30 seconds (arrows pointing up), and 250 mμmole of ADP was added at each point indicated by arrows pointing down. Rates of oxygen consumption are expressed in millimicroatoms per minute.

Although mitochondria from healthy oat plants readily oxidized succinate with RC ratios comparable to those obtained with this substrate with mitochondria from other plants (*9, 12*), they failed to oxidize reduced nicotinamide adenine dinucleotide (NADH), malate with or without NAD, malate plus pyruvate, or α-ketoglutarate. Preparations containing 20 m*M* malate also failed to oxidize succinate when an equal molar concentration of the latter was added. Bonner (*5*) reported that plant mitochondria that exhibit respiratory control oxidize succinate, NADH, and malate but not other Krebs cycle intermediates. Others (*12*) obtained preparations from avocado and sweet potato that utilized α-ketoglutarate with respiratory control. Whether slight differences in technique or inherent differences in plant materials are responsible for failure of oat mitochondria to exhibit the same oxidative properties as those from other plants has not yet been determined.

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Chromosomal Polymorphism in the White-Throated Sparrow, Zonotrichia albicollis (Gmelin)

Abstract. *In a study of 35 white-throated sparrows five distinct karyotypes were observed. A chromosomal polymorphism is present which involves at least two pairs of macrochromosomes. This species is phenotypically polymorphic with selective breeding occurring between morphs. Phenotype is related to chromosomal constitution, and selective breeding appears to maintain heterozygosity within the population.*

Distinct chromosomal polymorphism is rare in the subphylum Vertebrata (*1*), and none has previously been recorded in the class Aves. This report describes the chromosomal polymorphism found in the white-throated sparrow in Ontario.

The chromosomal complements of 35 individuals were obtained by growing, in primary culture (*2*), cells from kidneys, whole embryos, and feather pulp.

This sparrow has a typically avian karyotype containing 82 or 84 chromosomes. Easily recognizable macrochromosomes grade into small microchromosomes, the smallest of which are difficult to count even in good preparations. Chromosome 12, a small metacentric, is a useful marker; the chromosomes following it appear acrocentric, with the exception of pair 18, which is metacentric.

Figure 1 shows the first 24 chromosomes in the karyotypes of four individuals. In all the birds studied, the first pair of chromosomes is homomorphic and has a submedian centromere. However, the chromosomes making up the next four elements in the karyotype may vary. Three single chromosomes of equal lengths appear to be involved in this variation; they have arbitrarily been designated as 2, 3, and M. Chromosome 2 has a subterminal centromere, chromosome 3 is acrocentric, and chromosome M is almost strictly mediocentric. Five combinations of these chromosomes have been observed: 2–2–3–3 (bird A and 15 others), 2–3–3–M (bird B and 14 others), 2–2–3–M (bird C and two others), 2–2–2–M (only bird D), and 2–2–2–3 (only one bird, not illustrated). Chromosome 4 is the Z chromosome in this species; in females (birds A and B) a distinct W chromosome is present. The remaining macrochromosomes are common to all the birds studied.

There is no apparent relation between sex and the number of chromosomes 2, 3, and M that are present. Since chromosomes 2, 3, and M are of equal length and since they always total four in number, it appears probable that basically only two pairs of chromosomes are represented and that rearrangements, including pericentric inversions, may have caused the observed polymorphism. Birds of type A (2–2–3–3) may represent the original karyotype of this species. Autosomal polymorphism due to pericentric inversions has recently been reported in *Peromyscus maniculatus* (*3*) and in *Mastomys natalensis* (1). Studies on the pairing of homologs at meiosis will be necessary, however, before the true nature of the polymorphism in the white-throated sparrow will be known. In particular these studies should show whether the apparent nullisomy (3 and M), monosomy (2, 3, and M), and trisomy (2) are real.

The white-throated sparrow has been described on the basis of external morphology as a dimorphic species with selective breeding occurring between the morphs; it was also suggested that this breeding system might ensure heterosis in populations of the species (*4*). The exact relationship between the phenotype and the chromosomal constitution of the morphs is unknown. All birds bright in nuptial plumage have a single M chromosome and all birds dull in nuptial plumage lack this chromosome.

While it now appears that more than two morphs exist, there is no doubt that bright birds of either sex always mate with duller birds. Since in the wild the bright morphs never mate with other bright morphs, one would expect few, if any, animals to be homozygous for chromosome M. None has been found so far.

Fertile eggs can now be obtained in the laboratory, and karyotypic studies of family units as well as wild sibs should yield more refined data on this unique system of polymorphism.

In the white-throated sparrow, morphological variation, assortative mating,

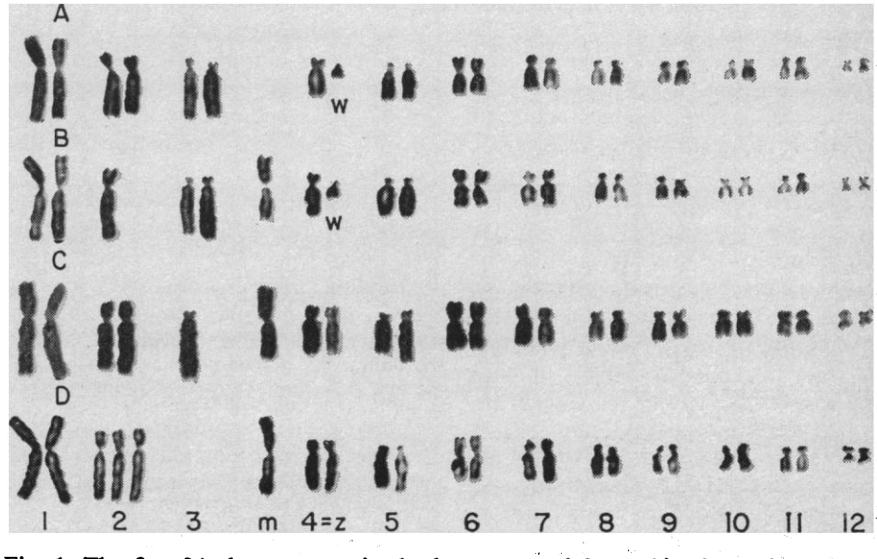

Fig. 1. The first 24 chromosomes in the karyotypes of four white-throated sparrows.