Could the Blood Parasite Leucocytozoon Deter Mallard Range Expansion?
Author(s): Dave Shutler, C. Davison Ankney, Darrell G. Dennis
Source: *The Journal of Wildlife Management*, Vol. 60, No. 3 (Jul., 1996), pp. 569-580
Published by: Allen Press
Stable URL: http://www.jstor.org/stable/3802074
Accessed: 04/12/2008 06:50

COULD THE BLOOD PARASITE *LEUCOCYTOZOON* DETER MALLARD RANGE EXPANSION?

DAVE SHUTLER,[1] Department of Zoology, University of Western Ontario, London, Ontario, N6A 5B7, Canada
C. DAVISON ANKNEY, Department of Zoology, University of Western Ontario, London, Ontario, N6A 5B7, Canada
DARRELL G. DENNIS, Canadian Wildlife Service, 153 Newbold Court, London, Ontario, N6E 1Z7, Canada

Abstract: We investigated whether the blood parasite *Leucocytozoon simondi* could slow mallard (*Anas platyrhynchos*) population growth in the east that has been associated with American black duck (*A. rubripes*; hereafter black duck) population decline. Susceptibility to parasites was compared among F1 ducklings produced from crosses between mallard and black ducks from areas of *Leucocytozoon* endemicity (Ontario), and between mallards from an area free of *Leucocytozoon* (Saskatchewan). We produced 6 "types" of ducklings: Ontario black duck × Ontario black duck (OB × OB), Ontario black duck × Ontario mallard (OB × OM), Ontario black duck × Saskatchewan mallard (OB × SM), OM × OM, OM × SM, and SM × SM. We predicted that because of probable coevolution of black ducks and *Leucocytozoon*, black duck ducklings would have resistance to the parasite. We also predicted that Ontario genes would confer some resistance to ducklings because these ducklings' parents had survived exposure to *Leucocytozoon*. In contrast, we predicted that mallard and Saskatchewan genes would not confer resistance, i.e., OB × OB ducklings would have greatest resistance to *Leucocytozoon*, SM × SM ducklings would have least, and remaining duckling types would have intermediate resistance. Of 169 ducklings exposed in 2 years in 3 geographically separate locales, none died, showed noticeable symptoms, or otherwise behaved abnormally. Nonetheless, weekly blood smears indicated that 91% of ducklings became infected, and many developed intense parasitemias. However, infection intensities were not different among the 6 duckling types. In addition, hematocrits were not lowered by intense infections. These results suggest that the effects of *Leucocytozoon* on wild waterfowl populations have been overestimated, and that *Leucocytozoon* will not prevent further range expansion of mallards.

J. WILDL. MANAGE. 60(3):569–580

Key words: American black ducks, *Anas platyrhynchos*, *Anas rubripes*, blood parasites, hybrids, *Leucocytozoon simondi*, mallard, Ontario, Saskatchewan.

Mallards appear to be displacing American black ducks in eastern North America through competition and/or introgressive hybridization (Rogers and Patterson 1984, Brodsky and Weatherhead 1984, Ankney et al. 1987, Merendino et al. 1993). Thus, continued mallard range expansion could lead to local or complete extinction of black ducks. However, the blood parasite *Leucocytozoon simondi* may be a barrier to this outcome (Khan and Fallis 1968, Dennis 1974). *Leucocytozoon* is endemic in much of the historic range of black ducks, and the parasite reportedly causes substantial duckling mortality (O'Roke 1934, Fallis et al. 1956, 1974; Barrow et al. 1968) that is higher among mallard than black duck ducklings (Khan and Fallis 1968). In this paper, we test whether differential susceptibility of mallards and black ducks to *Leucocytozoon* has the potential to prevent or slow mallard encroachment into those parts of the black duck range in which *Leucocytozoon* occurs.

Leucocytozoon is transmitted by black flies (Simuliidae) (Shewell 1955) and can infect 100% of mallards and black ducks in some populations in northeastern North America (O'Roke 1934, Nelson and Gashwiler 1941, Chernin 1952, Trainer et al. 1962, Bennett et al. 1974, 1975, 1991). In contrast, *Leucocytozoon* is rarely reported from central North America (Savage and Isa 1955, Burgess 1957). Differences in *Leucocytozoon* prevalence arise because, in the latter area, black fly vectors have less swift water breeding habitat and are often insufficiently numerous to spread the parasite (Herman 1968).

Historically, mallards occupied western and central North America, whereas black ducks occupied the eastern part of the continent (Bellrose 1976). This historical distribution pattern is consistent with current distributions of *Leucocy-*

[1] Present address: Canadian Wildlife Service, 115 Perimeter Road, Saskatoon, Saskatchewan, S7N 0X4, Canada.

tozoon (Khan and Fallis 1968). Because mallards have not coevolved with *Leucocytozoon* but are closely related to black ducks genetically (Ankney et al. 1986, Avise et al. 1990), they should be more susceptible to the parasite (Ewald 1983, 1993; Van Riper et al. 1986, Black 1992, Bennett et al. 1993).

If a *Leucocytozoon*-mediated barrier to mallards still exists, however, it is necessary to explain continuing mallard range expansion into areas where the parasite is endemic (Johnsgard and DiSilvestro 1976, Rusch et al. 1989, Merendino and Ankney 1994). One possibility is that mallards have acquired genes for *Leucocytozoon* resistance, either from introgressive hybridization with resistant black ducks, or via natural selection that has culled non-resistant mallards (collectively, the resistance hypothesis). A second possibility is that mallards are able to survive only in habitats within the boreal forest where *Leucocytozoon* is not present (e.g., areas without fast running water [the susceptibility hypothesis]). The null hypothesis is that a *Leucocytozoon*-mediated barrier does not and did not exist, and that no differences in susceptibility occur between duck species.

Our objective was to determine whether duckling mortality could limit mallard range expansion into areas of *Leucocytozoon* endemicity. We tested whether greater parasite resistance occurred among ducklings with a greater proportion of genes from presumably coevolved black ducks, or from parents recently exposed to *Leucocytozoon*; i.e., resistance of black ducks > mallards, and resistance of Ontario birds > resistance of Saskatchewan birds. Our protocol was to expose ducklings to black fly vectors of *Leucocytozoon* in seminatural conditions, and then observe ducklings for differences in mortality or signs of illness. We first ensured that sex, initial date of exposure, and site of exposure did not obscure infection patterns among duckling species and hybrids (collectively, "types").

We thank J. Haggeman for help at Lake St. Clair including taking care of ducks during our absences. A. Mullie, R. Bon, and G. Alderson provided field assistance, and colleague D. Hoysak shared duties associated with his and our concurrent studies. Team Cage Henge (J. Kominiek, P. Rutherford, S. Seiler, A. Mullie, D. Gagnier, B. and P. Wisenden, D. Hamilton, and R. Haggeman) helped build duck enclosures; P. Schleifenbaum and family accommodated ducks in 1992; S. Desser loaned equipment and with his crew provided guidance in the ways of *Leucocytozoon*; the staff of Canadian Wildlife Service in London provided logistical assistance (J. Robinson, N. North, and J. Sullivan); B. Clark of Canadian Wildlife Service in Saskatchewan sent us ducklings; A. Toline provided logistical assistance in Algonquin Park, and the Ontario Ministry of Natural Resources provided logistical assistance at Swan Lake (in particular, H. Anderson, J. Rice, D. Strickland, and M. Wilton). Drs. A. M. Fallis and R. A. Khan provided historical details of earlier studies, and commented on our conclusions. Funding was provided by the Natural Sciences and Engineering Research Council of Canada, the Black Duck Joint Venture, the Ontario Federation of Anglers and Hunters, and the University of Western Ontario.

METHODS

Initial Preparations

In April 1991, we advertised rewards to individuals finding active wild mallard and black duck nests in an area of *Leucocytozoon* endemicity in Ontario bounded approximately by Parry Sound in the west (80°0′W), North Bay in the north (46°30′N), Ottawa in the east (75°30′W), and Haliburton in the south (45°0′N). Eggs we collected from these nests were transported to Lake St. Clair, an area where vectors of *Leucocytozoon* are not found. In addition, we received 40 Saskatchewan mallard ducklings (Can. Wildl. Serv., Saskatchewan). Eggs were hatched in incubators, and we gave each duckling individually numbered web tags. Ducklings were sexed by cloacal inspection (Schemnitz 1980) within a day after hatching, and those that survived to 4 weeks were given individually numbered leg bands. Because we needed hybrids for our experiments on *Leucocytozoon,* we used these ducks for captive breeding rather than experiments. In 1992, we had 107 yearlings to breed for producing ducklings. We prepared yearlings for breeding by putting them in outdoor group pens in October so that they would form natural pair bonds (opposite-sex siblings were kept in separate pens). Up to 20 ducks were assigned to 1 of 6 group pens so that their pairings would produce ducklings hypothetically exhibiting the full range of *Leucocytozoon* resistance (Table 1). In early April, pairs were isolated in 2.4- × 1.5- × 0.9-m (8- × 5- × 3-ft) breeding pens with a nest box, ad libitum corn,

Table 1. Arrangement of ducks in group pens in 1992 at the Lake St. Clair National Wildlife Area. A similar arrangement was used in 1993.

Pen	M[a]	F[a]	Group size
1	10 OM (4)	10 OM (4)	20
2	9 OM (7)	4 SM (4), 5 OB (2)	18
3	7 OM (7)	7 OM (7)	14
4	9 SM (6)	4 SM (3), 5 OB (3)	18
5	9 OB (4)	10 OM (4)	19
6	9 OB (3)	4 SM (4), 5 OB (2)	18
Total	53 (16)	54 (15)	107

[a] OM denotes Ontario mallard; OB denotes Ontario black duck; SM denotes Saskatchewan mallard. No. of different clutches that account for each group is given in parentheses.

commercial duck food, and grit. Birds that had not formed pairs were assigned mates and similarly isolated. This procedure was repeated in 1993 (birds that bred in 1992 were paired with different mates), except that we also paired some yearlings produced in 1992.

We had unequal numbers of adult types to pair at the outset (Table 1). Furthermore, breeding success was uneven (Table 2). Hence, we had unequal numbers of each type of duckling for our experiments.

Methods for Testing Leucocytozoon Susceptibility.

To mimic conditions that wild ducklings would experience in contracting *Leucocytozoon*, we exposed them to vectors as early as possible in their development, and kept them exposed continuously for several weeks. Ducklings hatched asynchronously. At each of 5 consecutive weekly intervals, when they were between 2 and 9 days old, ducklings were transported from Lake St. Clair to our study areas in and around Algonquin Park, Ontario, Canada. This area continues to be a stronghold for black ducks, and mallards are relatively rare (K. Ross, Can. Wildl. Serv., pers. commun.). We numbered duckling "groups" by week of hatch (i.e., group I hatched in the first week in Jun, group II in the second week in Jun, etc.).

Experimental ducklings were housed in the same cages we had used for breeding. Cages had 2.5- × 2.5-cm mesh hardware cloth that allowed black flies free access to ducklings. We put cages on lake shores where black fly vectors are most abundant (Bennett 1960). To substitute for the warmth normally provided by brooding hens, we supplied ducklings with a heat lamp for the first 7 days that they were outdoors. Ducklings were maintained on an ad libitum diet, and were provided with a nest box for shelter. Initially, we kept up to 15 ducklings per cage. As ducklings became larger, the maximum was reduced to 8. Experimental ducklings were exposed continuously until the end of July.

Of the first 6 ducklings from a clutch, 1 of each sex was assigned to 1 of 2 experimental locales (see below) and a control group. For clutches producing fewer than 3 ducklings of 1 sex, we allocated individuals to experimental locales before allocating individuals to the control group. Controls were kept in outdoor cages at Lake St. Clair. For larger clutches, only 2 ducklings were retained as controls; the remainder was divided by sex and randomly assigned to our experimental locales. This protocol avoided results from separate locales that may have been biased because of differences in sex ratio or genetic origin of ducklings.

Because black fly population density and *Leucocytozoon* population virulence vary geographically (Herman et al. 1975, Desser and Ryckman 1976, Desser et al. 1978), results from 1 locale may not be applicable to other locales. Hence, in each year, we divided experimental

Table 2. Breeding success of pair types, and number of each type of duckling exposed or kept as controls.

	1992			1993		
M × F[a]	Pairs isolated	% of pairs producing viable ducklings	Ducklings exposed (controls)	Pairs isolated	% of pairs producing viable ducklings	Ducklings exposed (controls)
OB × OB	5	40	8 (2)	7	57	12 (3)
OB × OM, OM × OB	9	11	6 (1)	8	25	5 (0)
OB × SM, SM × OB	7	0	0 (0)	7	57	6 (2)
OM × OM	10	80	52 (7)	8	63	33 (8)
OM × SM, SM × OM	10	50	17 (2)	7	43	21 (5)
SM × SM	3	33	7 (1)	4	50	2 (2)
Totals	44	39	90 (13)	41	49	79 (20)

[a] OM denotes Ontario mallard; OB denotes Ontario black duck; SM denotes Saskatchewan mallard.

ducklings between locales that were more than 20 km apart (Lake Sasajewan and Kennisis Lake in 1992, Lake Sasajewan and Bena Lake in 1993). There was no electricity available for heat lamps at Bena Lake, so we kept ducklings outdoors at Lake Sasajewan for 7 days before transporting them to Bena Lake. Because wild hen mallards move their broods between lakes, this manipulation mimicked wild ducklings' experience.

Ducklings were visited at least every 2 days to be fed and checked for signs of parasitism. After a duckling is infected with *Leucocytozoon* sporozoites, 6 to 10 days elapse before mature gametocytes of the parasite appear in the blood stream (Desser 1967). To monitor infection rates, beginning at 10 days post-exposure, we took weekly blood smears from each experimental duckling's leg vein (Bennett 1970). A subsample of controls was also periodically checked for infection. Twenty-five microscope fields (roughly 6,000 red blood cells) of smears were examined under a microscope using a magnification of 400, and all mature *Leucocytozoon* gametocytes were counted. Infection intensities are expressed as number of parasites per 1,000 red blood cells. Because density of parasites subsides at night (Roller and Desser 1973), smears were made during the day. Nonetheless, in 1993 we recorded the exact time that samples were taken to confirm that time was not confounding our results.

In 1992, we sampled ducklings until 8 August. Hence, ducklings in group I were sampled more times than subsequent groups. A subsample of surviving ducks from 1992 was also sampled in spring 1993 for evidence of relapse, the surge of parasites in blood that serves to reinfect vector populations each year (Chernin 1952). Results from 1992 suggested that by the sixth weekly blood smear, infection intensities were minimal in more that 95% of cases. Hence, each duckling was sampled only 6 times in 1993.

Intensity of *Leucocytozoon* infection has been correlated with degree of anemia in domestic ducks (Kocan and Clark 1966, Maley and Desser 1977). To measure this symptom, in 1993 we took a microcapillary of blood from each duckling at the same time as we took blood smears. Capillaries were centrifuged and percent packed red blood cell volume (hematocrit) of each sample was calculated. Hematocrits of group III, IV, and V ducklings were not sampled 6 times because of logistic problems.

Maximum density of mature *Leucocytozoon* gametocytes occurs in ducklings' blood streams at about 10–24 days post-infection, and this is also when duckling mortality peaks (Desser 1967). Thus, the most biologically meaningful comparison is among ducklings that have been infected for these many days. However, negative blood smears do not necessarily imply that a duckling has not been infected, because low intensity parasitemias may not be detected in individual blood smears. Thus, we could never be certain of the initial date on which a duckling had become infected. The best we could do was to compare infection intensities of ducklings relative to the interval since they had been exposed. However, black fly populations fluctuate within and among years so that transmission dynamics vary according to Julian date on which a duckling hatches (and consequently when we added it to our experiments). Thus, we also compared course of infection relative to Julian date at which ducklings were exposed.

Genuine *Leucocytozoon*-negative smears may arise for 2 main reasons. First, ducklings may have a sterile immunity to the parasite; i.e., the duckling's immune system kills all parasites before they develop into gametocytes. Second, just by chance, a duckling may not have been bitten by, or received any *Leucocytozoon* from, infected black flies. In the first case, differences in sterile immunity are biologically meaningful and negatives should be included in comparisons. In the second case, negatives may add significant but biologically meaningless variation to results. Because we could not be certain whether causes of negatives were biologically meaningful, we analyzed data twice, once with and once without negatives.

Infection intensity data that included negatives were not normally distributed (Fig. 1, top, Kolmogorov-Smirnov tests, d's > 0.30, P's < 0.0001). Box-Cox tests (Krebs 1989) indicated that transformations would not improve fit to normality. Hence, except where sample sizes were large, we used non-parametric tests when analyzing these data. If negatives were excluded, data could be log-transformed to produce distributions that were closer to normality (Fig. 1 bottom, Kolmogorov-Smirnov tests, d's < 0.18, P's still <0.0001). We assume that parametric tests were sufficiently robust to deal with remaining departures from normality; at any rate, non-parametric tests gave qualitatively similar results.

We judged that a repeated measures analysis

of variance (ANOVA) should not be used on infection data that included negatives because the data were not normally distributed. However, if we excluded negatives, the resulting reduction in sample sizes prevented statistically meaningful comparisons. Hence, we included negatives but used non-parametric repeated measure ANOVA (Friedman tests). Because non-parametric tests are less powerful than parametric tests, we also present separate parametric results from each sampling interval to be certain that no differences were inadvertently overlooked. To derive appropriate statistical cutoff probabilities for these separate tests, we used a sequential Bonferroni cutoff criterion wherein the standard 0.05 cutoff probability is divided by the number of tests (Daniel 1978, Holm 1979, Rice 1989).

Most statistical analyses were performed on a Macintosh version of SYSTAT (1992). Power calculations for correlations were done by hand using formulae in Zar (1984). Sample sizes that we report vary because of breakage of hematocrit tubes during centrifugation, subsampling, predator-caused mortality (about 5%), and other reasons given above.

RESULTS

1. Mortality, Infection Intensity, and Hematocrit.—Eighty-three percent of ducklings in 1992 and 100% of ducklings in 1993 tested positive at least once for *Leucocytozoon*. However, no ducklings died as a result of *Leucocytozoon* infection (n = 169). Furthermore, we observed no symptoms that could be ascribed to *Leucocytozoon* in any duckling. In fact, we had only 1 duckling that became visibly sick and died. Although we could not determine its cause of death, we found no *Leucocytozoon* in this duckling 4 days before or on the day it died. No mortality from disease occurred among the control population either (n = 32), and we found no *Leucocytozoon* in 28 smears from controls (some individuals were sampled as many as 3 times, some were not sampled). Because we had no *Leucocytozoon*-caused mortality, the remainder of our results focus on infection intensity and hematocrit.

Infection intensities in 972 blood smears from experimental ducklings ranged from 0 to 67 parasites/1,000 blood cells (mean ± SD = 3.15 ± 6.73) (Fig. 1 top). Taking into account that these data include ducklings sampled after infection intensities had subsided, our mean is within the range of infection intensities reported elsewhere (e.g., Desser 1967, Khan and Fallis 1968).

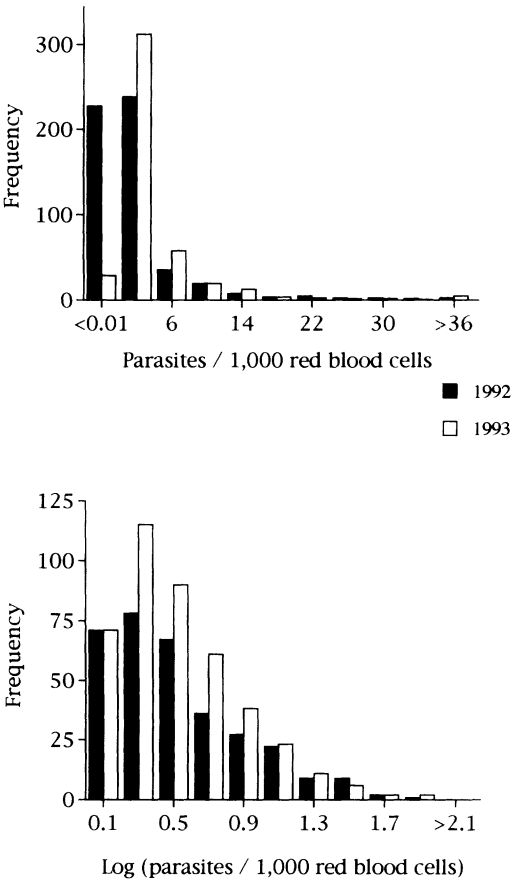

Fig. 1. Top. Distribution of infection intensities (all data combined) in 1992 (solid bars) and 1993 (open bars). Bottom. Log-transformed data with negatives excluded. Except for categories with "<" or ">", values on lower axes are midpoints.

Intensity assessments were highly repeatable based on a sample of smears that were known to have parasites (n = 98, r_s = 0.90, $P < 0.001$). We also tested whether an individual blood sample reliably reflected parasite loads. Paired smears taken from the same individual within an hour had similar parasite densities (r_s = 0.64, n = 10, P = 0.03). Hematocrit samples that were similarly paired were also significantly correlated (r_s = 0.59, n = 10, P = 0.04). Finally, in 1993, we tested whether our results could have been biased by time of day when samples were taken. We found no relation between time of day and infection intensity (all 1993 data combined, r = −0.01, n = 444, P = 0.78, power = 0.96) or time of day and hematocrit (all 1993

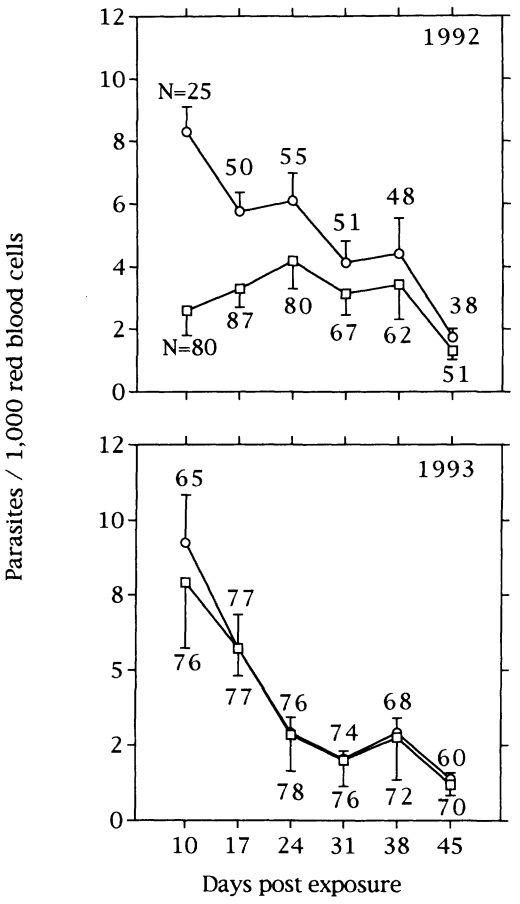

Fig. 2. Infection intensities at each sampling interval in 1992 (top) and 1993 (bottom). Upper data points in each graph do not include negatives whereas lower data points do. One standard error is shown for each point and sample sizes are given above or below associated error bars.

data, $r = 0.03$, $n = 384$, $P = 0.51$, power = 0.91). Furthermore, no non-linear patterns were visible in plotted data.

As reported elsewhere (Desser 1967), parasitemias reached a peak within the first 10–17 days (Fig. 2) and then declined to barely detectable levels (also see Tables 3 and 4 after 31 days post-exposure). If negatives are included, parasitemias peaked later in 1992 than in 1993 (Fig. 2). Otherwise, patterns of parasitemia were similar in each year.

Hematocrits ranged from 0.12 to 0.49. However, more intense *Leucocytozoon* infections were not associated with lower hematocrits (all 1993 data, $r = 0.05$, $n = 389$, power = 0.84, $P = 0.33$). An outlier individual whose hematocrit was 0.12 survived (the next lowest hematocrit recorded was 0.25).

2. *Sex.*—Females and males had similar intensity infections at each sampling interval whether or not negatives were included. Furthermore, the proportion of each sex that was infected in each sampling interval was the same (Chi-square tests, all P's > 0.10). Friedman tests on data from 1992 ($F = 0.71$, $n = 44$, $P = 0.40$) and 1993 ($F = 0.05$, $n = 69$, $P = 0.82$) also did not reveal any differences between the sexes in infection intensities. In 1993, hematocrits of females and males were similar at all sampling intervals, and a Friedman test also detected no differences ($n = 35$ for test up to and including 45 days post-exposure, $F = 0.92$, $n = 61$, $P = 0.34$; for test up to and including 31 days post-exposure, $F = 0.44$, $P = 0.51$).

3. *Site at Which Ducklings Were Exposed.*—In 1992, duckling parasitemias were lower and fewer ducklings became infected at Kennisis Lake than at Lake Sasajewan (Table 3). These differences persisted for the first 6 sampling intervals, and reappeared during relapse at 320 days post-exposure. When we excluded negatives, however, parasitemias were similar among locales (Table 3). When negatives are included, a Friedman test on data up to and including 45 days post-exposure revealed that differences were significant overall ($F = 108$, $n = 44$, $P < 0.001$).

In 1993, ducklings had higher intensity infections at Bena Lake than at Lake Sasajewan 10 days post-exposure (Table 3) whether or not negatives were included. After 10 days, no significant differences emerged. At no sampling interval did the proportion of infected ducklings differ between study sites (Chi-square tests, all P's > 0.10). A Friedman test indicated that infection intensities did not differ overall in 1993 between sites ($F = 0.25$, $n = 69$, $P = 0.62$).

Hematocrits were the same between sites based on the Bonferroni cutoff probability of 0.008. A Friedman test confirmed that there were no differences in hematocrits of ducklings according to the site at which they were exposed ($F = 0.01$, $n = 35$, $P = 0.91$).

4. *Date of Exposure.*—Comparison of progress of parasitemias among duckling groups in 1992 revealed different infection intensities at 38 days post-exposure; higher infection intensities of ducklings in group II accounted for this result. This result persisted when negatives were not included; ducklings in group II had more intense infections than their counterparts from groups I, III, and IV (Tukey's multiple comparison tests, P's < 0.05). In the remaining post-exposure intervals, infection intensities were

Table 3. Infection intensities (parasites/1,000 blood cells) by exposure site for each sampling interval in 1992–93.

Days post-exposure	Including negatives							Not including negatives						
	Alternate lake[a]			Lake Sasajewan				Alternate lake[a]			Lake Sasajewan			
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	P[b]	$\bar{x}$	SD	n	$\bar{x}$	SD	n	P[b]
							1992							
10	0.4	2.0	43	5.1	9.7	37	<0.001	9.3	2.6	2	8.2	11.3	23	0.38
17	0.9	2.6	46	6.0	6.8	41	<0.001	3.5	4.3	12	6.5	6.9	38	0.07
24	2.2	7.5	40	8.1	6.1	40	<0.001	5.3	11.0	17	6.5	8.1	38	0.20
31	1.6	3.7	35	4.9	6.9	32	<0.001	2.9	4.7	19	4.9	6.9	32	0.08
38	1.4	2.8	32	5.6	12.1	30	<0.001	2.5	3.4	18	5.6	12.1	30	0.15
45	0.9	2.4	24	1.6	1.5	27	<0.001	1.9	3.3	12	1.7	1.5	26	0.42
52	1.0	2.4	14	1.1	0.8	17	0.02	2.4	3.3	6	1.2	0.8	16	0.41
59	0.6	0.8	8	0.7	0.9	9	0.55	1.1	0.8	4	0.9	0.9	7	0.61
320	0.1	0.1	24	0.2	0.2	24	<0.001	0.2	0.1	5	0.3	0.2	16	0.36
							1993							
10	11.4	14.1	35	4.9	10.0	41	<0.001	12.5	14.3	32	6.1	10.9	33	<0.001
17	4.8	5.9	35	6.4	4.8	42	0.62	4.8	5.9	35	6.4	12.3	42	0.53
24	2.1	3.9	35	3.4	4.7	43	0.02	2.3	4.0	33	3.4	4.7	43	0.07
31	2.0	2.1	35	2.0	2.5	41	0.70	2.0	2.1	34	2.0	2.5	40	0.77
38	3.0	4.1	35	2.5	3.9	37	0.51	3.2	4.1	33	2.6	4.0	35	0.48
45	0.7	0.7	35	1.6	2.1	35	0.02	0.9	0.7	28	1.8	2.1	32	0.04

[a] Kennisis Lake in 1992, Bena Lake in 1993.

[b] For comparisons that include negatives, probabilities are based on Mann-Whitney U-tests on untransformed data. For comparisons that do not include negatives, probabilities are based on t-tests on log-transformed data. Significant Bonferroni cutoff probability is 0.006 for 1992, and 0.008 for 1993.

similar regardless of when a duckling was exposed. Furthermore, no differences among groups persisted when ducklings were sampled the following spring at 320 days post-exposure. A Friedman test also suggested that date of exposure had no significant effect on average infection intensities from the entire season (F = 1.15, n = 53, P = 0.35).

In 1993, differences among groups in infection intensities occurred at 24, 38, and 45 days post-exposure. When we excluded negatives, significant differences were detected only at 24 days post-exposure, when groups IV and V had higher infection intensities than groups I, II, and III (Tukey tests, P's < 0.05). At 38 days post exposure, group II had higher infection inten-

Table 4. Infection intensities (parasites/1,000 blood cells) by duckling type at each sampling interval in 1992–93.

Days post-exposure	Duckling type[a]																P[b] when negatives	
	OB × OB			OB × OM			OB × SM			OM × OM			OM × SM			SM × SM			
	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	Incl.	Not incl.
										1992										
10	3.0	6	3	7.1	6	2				1.8	45	9	2.0	14	7	4.6	7	4	0.18	0.84
17	5.2	7	4	0.9	6	4				4.0	51	31	1.7	16	7	1.7	7	4	0.66	0.60
24	1.0	6	3	6.3	6	5				4.4	44	33	5.5	17	10	0.8	7	4	0.34	0.44
31	1.0	6	4	1.6	5	4				3.9	37	29	3.6	14	11	0.8	5	3	0.69	0.74
38	0.8	4	3	1.0	6	4				2.5	36	28	1.3	9	26	14.3	7	6	0.10	<0.001
45	1.9	4	4	1.5	6	4				1.0	29	19	1.4	6	6	2.2	6	5	0.20	0.52
52	0.0	1	0	0.6	6	0				1.3	20	15				0.8	4	3	0.40	0.15
320	0.1	5	2	0.4	2	0				0.1	27	10	0.2	7	4	0.2	7	4	0.23	0.72
										1993										
10	7.3	12	11	0.8	5	5	7.9	5	4	6.0	32	23	11.2	20	20	26.2	2	2	<0.001	0.03
17	6.2	12	12	2.2	5	5	2.6	5	5	8.1	32	32	3.2	21	21	7.1	2	2	0.10	0.18
24	2.4	12	12	1.8	5	5	1.8	5	5	4.2	33	32	1.5	21	20	3.3	2	2	0.31	0.28
31	1.5	12	12	0.6	5	4	1.0	5	5	2.8	31	30	1.7	21	21	1.5	2	2	0.19	0.16
38	1.2	12	11	2.1	5	4	4.3	5	4	2.9	28	27	3.4	21	21	0.7	1	1	0.04	0.25
45	0.8	12	10	0.8	5	4	0.6	5	4	1.1	26	23	1.7	21	18	1.8	1	1	0.53	0.38

[a] No OB × SM ducklings were produced in 1992. Negatives are included in means. n_1 denotes sample sizes including negatives, n_2 denotes sample sizes without negatives.

[b] P values are based on Kruskal-Wallis tests when negatives are included, and ANOVAs when negatives are not included. Significant Bonferroni cutoff probability is 0.006 for 1992, and 0.008 for 1993. Power for tests that do not include negatives ranged from 0.31–0.52 for cases where Ps > 0.05.

Table 5. Hematocrits by duckling type at each sampling interval in 1993.

Days post exposure	Duckling type[a]																		P^b when negatives	
	OB × OB			OB × OM			OB × SM			OM × OM			OM × SM			SM × SM				
	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	$\bar{x}$	n_1	n_2	Incl.	Not incl.
10	0.34	12	11	0.34	3	3	0.35	5	5	0.35	32	23	0.33	20	20	0.34	1	1	0.29	0.25
17	0.38	12	12	0.35	5	5	0.41	5	5	0.38	31	31	0.39	21	21	0.36	2	2	0.16	0.16
24	0.38	11	11	0.38	4	4	0.41	5	5	0.40	27	26	0.38	20	19	0.39	1	1	0.12	0.14
31	0.38	12	12	0.36	5	4	0.37	5	5	0.39	28	27	0.37	19	19	0.31	1	1	0.21	0.32
38	0.38	9	8	0.36	5	4	0.37	4	3	0.38	21	20	0.36	19	19	0.29	1	0	0.33	0.26
45	0.39	3	3	0.36	4	3	0.39	3	3	0.40	14	12	0.37	19	18				0.66	0.71

[a] Means include negatives. n_1 denotes samples when negatives are included, n_2 denotes samples when negatives are excluded.
[b] P values are based on ANOVAs. Types for which $n = 1$ were not included in tests. Significant Bonferroni cutoff probability is 0.008. Power ranged from 0.16–0.64.

sities than group III (Tukey test, $P = 0.02$). However, mean infection intensities were the same among groups in 1993 (Friedman test excluding group V because of insufficient data, $F = 2.12$, $n = 69$, $P = 0.11$).

Hematocrits were similar among groups except at 17 days post-exposure, when group II had higher hematocrits than groups I and III, and group IV had higher hematocrits than group III (Tukey tests, P's < 0.05) whether or not negatives were included. We restricted our repeated measures test to the first 4 post-exposure intervals to include all groups, and found no differences in hematocrits (Friedman test excluding group V, $F = 1.66$, $n = 61$, $P = 0.19$).

5. *Duckling Type.*—Our principal objective was to measure differences in *Leucocytozoon* susceptibility among different duckling types. In 1992, no significant differences were observed in infection intensities except at 38 days post-exposure if negatives were excluded (Table 4); in this single instance, pure Saskatchewan mallard ducklings had the highest intensity infections of duckling types. Differences in infection intensities among duckling types were not significant overall (up to and including 45 days post-exposure, Friedman test, $F = 0.23$, $n = 44$, $P = 0.92$). Because of small sample sizes for some types, we repeated these tests using only pure Ontario mallard ducklings and Ontario × Saskatchewan mallard ducklings, but still found no significant differences (Friedman test up to and including 45 days post-exposure, $F = 0.10$, $n = 31$, $P = 0.76$).

In 1993, infection intensities differed among duckling types at 10 days post-exposure, but this difference was not significant if negatives were excluded (Table 4). Pure Saskatchewan mallards and Ontario mallard × Saskatchewan mallard ducklings had higher intensity infections, but no significant differences were detected (Tukey tests on data excluding negatives, all P's > 0.07). A Friedman test also indicated a relation between duckling type and *Leucocytozoon* susceptibility (pure Saskatchewan mallards excluded because of insufficient data, $F = 3.19$, $n = 68$, $P = 0.02$). Although these results suggested some support for the susceptibility hypothesis, the data (Table 4) indicate no clear relation between predicted susceptibility and infection intensity. Because of small sample sizes for some types, we repeated a Friedman test on pure Ontario black duck, pure Ontario mallard, and Ontario × Saskatchewan mallard ducklings, but again found no significant differences (up to and including 45 days post-exposure, $F = 0.81$, $n = 58$, $P = 0.45$).

Hematocrits did not differ among duckling types at any post-exposure interval in 1993, whether or not negatives were included (Table 5). Despite this, hematocrits differed overall among duckling types (Friedman test excluding Saskatchewan mallards, $F = 4.15$, $n = 35$, $P = 0.01$). If we included only the 3 duckling types used in the infection intensity analysis above, differences among types were not as extreme (Friedman test up to 38 days post-exposure, $F = 2.62$, $n = 43$, $P = 0.09$).

DISCUSSION

Substantial mortality from *Leucocytozoon* frequently has been reported among waterfowl (see Desser and Bennett 1993 for a review). However, many of these reports are based on mortality in domestic stocks of mallards (e.g., Chernin 1952, Fallis et al. 1956). Many other reports are based on experimental injection of *Leucocytozoon* into ducks (e.g., Anderson et al. 1962, Desser 1967). Hence, results from these studies should not be extrapolated to wild pop-

ulations naturally exposed to *Leucocytozoon*. In fact, although many species of birds can carry *Leucocytozoon* (Desser and Bennett 1993, Bennett et al. 1993), only rarely are there reports of mortality from *Leucocytozoon* in wild birds (Khan and Fallis 1968, Bennett et al. 1993). It could be argued that ad libitum diets enhanced the survival of our ducklings. However, domestic ducks on ad libitum diets suffer substantial mortality (references above). Hence diet is not a complete explanation for the absence of mortality we observed relative to the 31% mortality Khan and Fallis observed in their wild ducklings. In fact, most observations of parasites in wild birds suggest that absence of mortality is to be expected (Bennett et al. 1993).

That any mortality of wild ducks was observed in the Khan and Fallis study is thus surprising, and requires some explanation. One possibility is that rearing conditions for their ducklings were different from ours; for example Khan and Fallis (1968) did not use brood lamps when keeping ducklings outdoors (A. Murray Fallis, Caledon, Ont., and Russ Khan, Dep. Biol., Memorial Univ. Newf., pers. commun. 1995). Another possibility is that *Leucocytozoon* populations were more virulent in the years of their study. Parasite virulence can increase substantially when host density is sufficient to maintain transmission rates (Herre 1993). At the time of the Khan and Fallis study, research on *Leucocytozoon* had been ongoing at Lake Sasajewan for 20 years, and captive ducks had been maintained at much higher densities than occurs in this area in the wild. Thus, it is possible that *Leucocytozoon* had been selected for increased virulence during this interval. The explanation for the differences in mortality between this and the previous study is uncertain.

Another aspect of Khan and Fallis (1968) report is more significant. They reported that 40% of 25 mallard and 18% of 17 black duck ducklings died from *Leucocytozoon*. However, this difference, although statistically significant, is based on a total of 42 ducklings. There are numerous possible explanations for this difference other than differential susceptibility of mallards and black ducks. For instance, the differences they observed may have been the result of a type I statistical error. Second, their ducklings came from game farms, and the number of generations the population had been in captivity was unknown (Fallis and Khan, pers. commun.). This history could easily have made these birds more susceptible to parasites than completely wild populations (Bennett et al. 1993). Furthermore, number of broods used in their study was not known (Fallis and Khan, pers. commun.), so it is possible that their results were confounded by a few highly related individuals. If either of these was the case, the Khan and Fallis (1968) results might be ascribed to susceptible genetic stocks. A third possibility is that hybridization events between mallards and black ducks in the last 30 years have integrated genes for *Leucocytozoon* resistance into the mallard gene pool, including Saskatchewan. This homogenization of the mallard gene pool may have occurred because of substantial movements of males from their natal areas to other breeding areas. Indeed, modern black duck populations appear genetically indistinguishable from mallards (Ankney et al. 1986, Avise et al. 1990). Further investigation would be necessary to distinguish among these possibilities.

Even though we observed no mortality, more subtle differences in susceptibility to *Leucocytozoon* could possibly affect persistence of mallard populations in northeastern North America. For example, more intense infections could slow growth rates, weaken individuals so that they were more susceptible to predators, decrease their ability to invest in secondary sex characters that are used to attract mates (Hamilton and Zuk 1982), etc. Hence, we also compared symptoms of infection among ducklings, including hematocrits and infection intensity. Intensity of *Leucocytozoon* infection has been correlated with degree of anemia in domestic ducks (Kocan and Clark 1966, Maley and Desser 1977). However, we found no relation between infection intensity and hematocrit, despite the fact that our ducklings had infection intensities similar to those observed in domestic ducklings in previous studies. Although we did detect differences among duckling types in hematocrit, because hematocrit was not affected by *Leucocytozoon*, this result is of no importance to our experiments. It would appear that wild populations of ducks are able to maintain normal blood chemistry even when 7% of their erythrocytes are occupied by *Leucocytozoon*. The ability to maintain normal hematocrit also suggests that *Leucocytozoon* is more benign to wild ducks than has been suggested by research based primarily on domestic ducks.

We observed some differences in *Leucocytozoon* prevalence among sites. In particular, Kennisis Lake ducklings took longer to obtain infections than their counterparts at Lake Sa-

sajewan. The Kennisis Lake site was more exposed to wind than the Lake Sasajewan site, and wind may have reduced black fly attacks on ducklings. In addition, Kennisis Lake is surrounded by several recent cottage developments, whereas the area around Lake Sasajewan has remained essentially unaltered for several decades. Hence, human alterations of Kennisis Lake habitat may have reduced the black fly population relative to that of Lake Sasajewan. If black fly populations were denser at Lake Sasajewan, higher intensity infections found here among infected ducklings suggest either that *Leucocytozoon* was more virulent at Lake Sasajewan, or that more black fly bites result in higher parasitemias than do fewer bites. Our efforts to census black fly population densities were unsuccessful, so we have no firm data on the possibility of differences in black fly densities among our study sites. However, differences in infection rate and black fly biting rate have been observed between 2 sites in Algonquin Park (Khan, pers. commun.). Hence, as might be expected, certain locales appeared to be safer havens from *Leucocytozoon* than others, and if the parasite is important, we might expect ducks to make use of these areas.

We also observed, in the second year of the study, that ducklings on Bena Lake had higher intensity infections 10 days post exposure than those at Lake Sasajewan. Because infections take 6 to 10 days before they produce mature gametocytes in the blood stream, Bena Lake ducklings probably acquired their infections in the week the ducklings spent at Lake Sasajewan. Ducklings at Bena Lake were sampled 4 days after they had been moved from Lake Sasajewan, and this stress may have played a role in temporarily increasing their parasitemias (Applegate 1970). Stress from moving between ponds when led by a hen is probably less pronounced than what we caused, and probably has a minimal effect on parasitemias, so the result we obtained may not be of significance to wild populations.

Time of year at which ducklings were exposed influenced when they became infected, especially in 1993. If duckling mortality from *Leucocytozoon* is important, hen mallards could time laying of their clutches to coincide with seasons that have, on average, reduced *Leucocytozoon* transmission. When black flies first emerge, they carry no *Leucocytozoon*. They need to first feed on infected ducks to become infected themselves. Before ducklings hatch, duck populations in our study area are at low densities, and the chance of a black fly obtaining *Leucocytozoon* is fairly low. Once ducklings hatch, host density increases dramatically, and a greater proportion of black flies picks up parasites. Thus, there is a lag time between first black fly emergence and peak in *Leucocytozoon* transmission (Desser, Univ. Toronto, pers. commun.). Hence, on average, ducklings would have more time to develop without *Leucocytozoon* if they hatched earlier in the year. However, countervailing selection may select for laying dates that are more closely associated with food availability.

Our most important tests involved comparing susceptibility of duckling types to *Leucocytozoon*. Bennett et al. (1993) pointed out that birds introduced to unfamiliar areas often suffer greater mortality than endemic populations. We observed significantly greater infection intensities in 2 mallard types in 1993 relative to the single black duck type, and this is consistent with Bennett et al. observations. However, differences were not significant after 10 days post-exposure. Furthermore, trends were not the same in 1992. Consequently, we conclude that duckling types differ little or not at all in their susceptibility to *Leucocytozoon*. Thus, we have no support for either the resistance or the susceptibility hypotheses; therefore we accept the null hypothesis.

There are at least 2 other hypotheses that we can consider with our data. One part of our protocol was to create hybrids between 2 species of ducks. The biological species concept (Mayr 1963) and Barton and Hewitt (1985) hybrid zone model both predict that hybrids will be more susceptible to parasites than pure parental stocks. Support for this prediction has been found in studies of various animal and plant taxa (Sage et al. 1986, Dupont and Crivelli 1988, Whitham 1989, Moulia et al. 1991, Bert et al. 1993). However, the opposite pattern of "hybrid vigor" has also been observed (Boecklen and Spellenberg 1990), and equivalent susceptibility has been observed between parental and hybrid stocks (Heaney and Timm 1985). Our data fall into the latter category, because 2 types of hybrid ducklings we used were no more susceptible to *Leucocytozoon* than pure types. Generalizations about how hybridization affects susceptibility to disease and fitness in general may be premature (Arnold and Hodges 1995), and may

be hampered by species and hybrid definitions that do not apply to all situations.

MANAGEMENT IMPLICATIONS

Leucocytozoon may have been viewed as a "passive" management tool for preventing mallard incursion into black duck habitat. However, our results suggest that *Leucocytozoon* will neither be a barrier to additional gene flow between mallards and black ducks, nor will it prevent mallards from occupying areas currently occupied solely by black ducks. This implies that the future of the black duck will continue to be a source of controversy and concern (Ankney et al. 1987, 1989; Conroy et al. 1989, Rusch et al. 1989, Merendino et al. 1993).

LITERATURE CITED

ANDERSON, J. R., D. O. TRAINER, AND G. R. DEFOLIART. 1962. Natural and experimental transmission of the waterfowl parasite, *Leucocytozoon simondi* Mathis and Leger, in Wisconsin. Zoo. Res. 1:155–164.

ANKNEY, C. D., D. G. DENNIS, AND R. C. BAILEY. 1987. Increasing mallards, decreasing American black ducks: coincidence or cause and effect? J. Wildl. Manage. 51:523–529.

———, ———, AND ———. 1989. Increasing mallards, decreasing American black ducks: no evidence for cause and effect: a reply. J. Wildl. Manage. 53:1072–1075.

———, ———, L. N. WISHARD, AND J. E. SEEB. 1986. Low genic variation between black ducks and mallards. Auk 103:701–709.

APPLEGATE, J. E. 1970. Spring release of *Plasmodium relictum* infections in an experimental field population of English sparrows (*Passer domesticus*). J. Wild. Dis. 6:37–42.

ARNOLD, M. L., AND S. A. HODGES. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. 10:67–71.

AVISE, J. C., C. D. ANKNEY, AND W. S. NELSON. 1990. Mitochondrial gene trees and the evolutionary relationship of mallards and black ducks. Evolution 44:1109–1119.

BARROW, J. H., N. KELKER, AND H. MILLER. 1968. The transmission of *Leucocytozoon simondi* to birds by *Simulium rugglesi* in northern Michigan. Am. Midl. Nat. 79:197–204.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

BELLROSE, F. G. 1976. Ducks, geese, and swans of North America. Stackpole Books, Harrisburg, Penn. 544pp.

BENNETT, G. F. 1960. On some ornithophilic blood-sucking diptera in Algonquin Park, Ontario, Canada. Can. J. Zool. 38:377–389.

———. 1970. Simple techniques for making avian blood smears. Can. J. Zool. 48:585–586.

———, W. BLANDIN, H. W. HEUSMANN, AND A. G. CAMPBELL. 1974. Hematozoa of the Anatidae of the Atlantic Flyway. 1. Massachusetts. J. Wildl. Dis. 10:442–451.

———, M. A. PIERCE, AND R. W. ASHFORD. 1993. Avian haematozoa: mortality and pathogenicity. J. Nat. Hist. 27:993–1001.

———, A. D. SMITH, W. WHITMAN, AND M. CAMERON. 1975. Hematozoa of the Anatidae of the Atlantic flyway. II. The Maritime provinces of Canada. J. Wildl. Dis. 11:280–289.

———, V. D. STOTTS, AND M. C. BATEMAN. 1991. Blood parasites of black ducks and other anatids from Labrador and insular Newfoundland. Can. J. Zool. 69:1405–1407.

BERT, T. M., D. M. HESSELMAN, W. S. ARNOLD, W. S. MOORE, H. CRUZ-LOPEZ, AND D. C. MARELLI. 1993. High frequency of gonadal neoplasia in a hard clam (*Mercenaria* spp.) hybrid zone. Mar. Biol. 117:97–104.

BLACK, F. L. 1992. Why did they die? Science 258: 1739–1740.

BOECKLEN, W. J., AND R. SPELLENBERG. 1990. Structure of herbivore communities in two oak (*Quercus* spp.) hybrid zones. Oecologia 85:92–100.

BRODSKY, L. M., AND P. J. WEATHERHEAD. 1984. Behavioral and ecological factors contributing to American black duck-mallard hybridization. J. Wildl. Manage. 48:846–852.

BURGESS, G. D. 1957. Occurrence of *Leucocytozoon simondi* Mathis and Leger in wild waterfowl in Saskatchewan and Manitoba. J. Wildl. Manage. 21:99–100.

CHERNIN, E. 1952. The epizootiology of *Leucocytozoon simondi* infections in domestic ducks in northern Michigan. Am. J. Hyg. 56:39–57.

CONROY, M. J., G. R. BARNES, R. W. BETHKE, AND T. D. NUDDS. 1989. Increasing mallards, decreasing American black ducks—no evidence for cause and effect: a comment. J. Wildl. Manage. 51:523–529.

DANIEL, W. W. 1978. Applied nonparametric statistics. Houghton Mifflin, Boston, Mass.

DENNIS, D. G. 1974. Waterfowl observations during the nesting season in Precambrian and clay belt areas of north-central Ontario. Can. Wildl. Serv. Rep. Ser. 29:53–56.

DESSER, S. S. 1967. Schizogony and gametogony of *Leucocytozoon simondi* and associated reactions in its avian host. J. Protozool. 14:244–254.

———, AND G. F. BENNETT. 1993. The genera *Leucocytozoon*, *Haemoproteus* and *Hepatocystis*. Pages 273–305 *in* Kreier, J. P., ed. Parasitic protozoa. Vol. 4. Acad. Press, London, U.K.

———, AND A. K. RYCKMAN. 1976. The development and pathogenesis of *Leucocytozoon simondi* in Canada and domestic geese in Algonquin Park, Ontario. Can. J. Zool. 54:634–643.

———, J. STUHT, AND A. M. FALLIS. 1978. Leucocytozoonosis in Canada geese in upper Michigan I. Strain differences among geese from different localities. J. Wildl. Dis. 14:124–131.

DUPONT, F., AND A. J. CRIVELLI. 1988. Do parasites confer a disadvantage to hybrids? Oecologia 75: 587–592.

EWALD, P. W. 1983. Host-parasite relations, vec-

tors, and the evolution of disease severity. Annu. Rev. Ecol. Syst. 14:465–485.

———. 1993. Evolution of infectious disease. Oxford Univ. Press, Oxford, U.K. 256pp.

FALLIS, A. M., R. C. ANDERSON, AND G. F. BENNETT. 1956. Further observations on the transmission and development of *Leucocytozoon simondi*. Can. J. Zool. 34:389–404.

———, S. S. DESSER, AND R. A. KHAN. 1974. On species of *Leucocytozoon*. Adv. Parasitol. 12:1–67.

HAMILTON, W. D., AND M. ZUK. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

HEANEY, L. R. AND R. M. TIMM. 1985. Morphology, genetics, and ecology of pocket gophers (genus *Geomys*) in a narrow hybrid zone. Biol. J. Linn. Soc. 25:301–316.

HERMAN, C. M. 1968. Blood parasites of North American waterfowl. Trans. North Am. Wildl. Nat. Resour. Conf. 33:348–359.

———, J. BARROW JR., AND I. B. TARSHIS. 1975. Leucocytozoonosis in Canada geese at the Seney National Wildlife Refuge. J. Wildl. Dis. 11:404–411.

HERRE, E. A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. Science 259:1442–1445.

HOLM, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6:65–70.

JOHNSGARD, P. A., AND R. DISILVESTRO. 1976. Seventy-five years of changes in mallard-black duck ratios in eastern North America. Am. Birds 30: 905–908.

KHAN, R. A., AND A. M. FALLIS. 1968. Comparison of infections with *Leucocytozoon simondi* in black ducks (*Anas rubripes*), mallards (*Anas platyrhynchos*), and white Pekins (*Anas bochas*). Can. J. Zool. 46:773–780.

KOCAN, R. M., AND D. T. CLARK. 1966. Anemia in ducks infected with *Leucocytozoon simondi*. J. Protozool. 13:465–468.

KREBS, C. J. 1989. Ecological methodology. Harper and Row, New York, N.Y., 654pp.

MALEY, G. J. M., AND S. S. DESSER. 1977. Anemia in *Leucocytozoon simondi* infections. I. Quantification of anemia, gametocytemia, and osmotic fragility of erythrocytes in naturally infected Pekin ducklings. Can. J. Zool. 55:355–358.

MAYR, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, Mass. 797pp.

MERENDINO, M. T., AND C. D. ANKNEY. 1994. Habitat use by Mallards and American Black ducks breeding in central Ontario. Condor 96:411–421.

———, ———, AND D. G. DENNIS. 1993. Increasing mallards, decreasing American black ducks: more evidence for cause and effect. J. Wildl. Manage. 57:199–208.

MOULIA, C., J. P. AUSSEL, F. BONHOMME, P. BOURSOT, J. T. NIELSEN, AND F. RENAUD. 1991. Wormy mice in a hybrid zone: a genetic control of susceptibility to parasite infection. J. Evol. Biol. 4:679–687.

NELSON, E. C., AND J. S. GASHWILER. 1941. Blood parasites of some Maine waterfowl. J. Wildl. Manage. 5:199–205.

O'ROKE, E. C. 1934. A malaria-like disease of ducks caused by *Leucocytozoon anatis* Wickware. Univ. Michigan School For. and Conserv. Bull. 4:1–44.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

ROGERS, J. P., AND J. H. PATTERSON. 1984. The black duck population and its management. Trans. North Amer. Wildl. Natur. Resour. Conf. 49: 527–534.

ROLLER, N. F., AND S. S. DESSER. 1973. Diurnal periodicity in peripheral parasitemias in ducklings (*Anas boschas*) infected with *Leucocytozoon simondi* Mathis and Leger. Can. J. Zool. 51:1–9.

RUSCH, D. H., C. D. ANKNEY, H. BOYD, J. R. LONGCORE, F. MONTALBANO, J. K. RINGELMAN, AND V. D. STOTTS. 1989. Population ecology and harvest of the American black duck: a review. Wild. Soc. Bull. 176:379–406.

SAGE, R. D., D. HEYNEMAN, K.-C. LIM, AND A. C. WILSON. 1986. Wormy mice in a hybrid zone. Nature 324:60–63.

SAVAGE, A., AND J. M. ISA. 1955. An occurrence of *Leucocytozoon simondi* in Manitoba. Can. J. Microbiol. 1:291–292.

SCHEMNITZ, S. D., EDITOR. 1980. Wildlife management techniques manual. Fourth ed. The Wildl. Soc., Washington, D. C. 686pp.

SHEWELL, G. E. 1955. Identity of the black fly that attacks ducklings and goslings in Canada (Diptera: Simuliidae). Can. Entomol. 87:345–349.

SYSTAT. 1992. SYSTAT: statistics. Version 5. SYSTAT Inc. Evanston, Ill., 724pp.

TRAINER, D. O., C. S. SCHILDT, R. A. HUNT, AND L. R. JAHN. 1962. Prevalence of *Leucocytozoon simondi* among some Wisconsin waterfowl. J. Wildl. Manage. 26:137–143.

VAN RIPER, C. V. III, S. G. VAN RIPER, M. L. GOFF, AND M. LAIRD. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol. Monogr. 56:327–344.

WHITHAM, T. G. 1989. Plant hybrid zones as sinks for pests. Science 244:1490–1493.

ZAR, J. H. 1984. Biostatistical analysis. Second ed. Prentice-Hall, Englewood Cliffs, N.J. 718pp.

Received 10 April 1995.
Accepted 23 January 1996.
Associate Editor: Murphy.