Crested Mutant in Zebra Finches

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The crested mutant in zebra finches was shown statistically to be an autosomal codominant lethal. Homozygous crests likely die as embryos. Even the heterozygous crests were deficient in numbers, allowing the conclusion that heterozygous crest in zebra finches is also detrimental (subvital), or it has reduced penetrance.

Keywords: Zebra finch, *Taeniopygia guttata*, Crested mutant, Crest, Lethal

Introduction

The wild-type zebra finch, *Taeniopygia guttata* (previously *Poephila guttata*), (Figures 1 and 2) has a smooth cap of feathers from the beak over the top of the head (Figure 3). Crested zebra finches often have a flattish, or somewhat raised rosette, nearly a centimeter in diameter on the top of the head (Figure 4). Sometimes the crest is reduced in size and the feathers stand more erect, so that the crest does not resemble a complete rosette (Figure 5). Thus, there can be some variable expressivity.

The zebra finch's crested mutant gene is a dominant gene (Zann, 1996). Any outcross of a crested zebra finch to a normal yields some crested offspring. And crested offspring have not resulted from non-crested parents. However, the mutant could be a codominant lethal (Miller, 1996 and 1997), as in canaries or certain breeds of domestic ducks (general knowledge with canary and duck breeders). When crested zebra finches became available, the question naturally arose as to whether inheritance in crested zebra finches was like the inheritance in the crested canary and crested ducks.

Materials and Methods

The senior author has kept a few breeding pairs of the zebra finch at his home since 1985. Most of the original non-crested stock came from Garrie Landry, Louisiana (Miller, 1978). The crested zebra finches came from The Ark, a pet store in Ames, Iowa, in the year 1999.

Most of the diet was small grains, mostly millets and some niger thistle and canary seeds. An important vitamin and protein supplement was LeFebres granules for finches. Both a granular salt misture fortified with trace elements and calcium source, such as granular limestone size F, were always available.

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Figure 1: Wild-Type Male Zebra Finch

Figure 2: Wild-Type Female Zebra Finch

Figure 3: Head of Wild-Type Female Zebra Finch

Figure 4: Head of Crested Zebra Finch (Good Crest)

Figure 5: Head of Crested Zebra Finch (Irregular Crest)

Each breeding pair had its own cage (24 × 14 × 14 inches) to eliminate the possibility of infidelity. Each cage had two disposable nests with one near each end of the cage. This gave the birds a choice of nest sites (Morris, 1954). Nest material included dry white pine needles, fine grass leaves and stems. Burlap sacking squares were pinned to the side of the cage; the birds plucked the strands and carried them to the nest.

Many eggs showed no development and dead embryos from environmental reasons were frequent. Eggs might be buried from excess nest material. Eggs might be left too cold during incubation. Lacking easy access to microscopes and considering the difficulty of examining the tiny dead embryos for possible brain hernias, we decided that straightforward statistical use of testcrosses of possible crested homozygotes was just as useful. Crested finches were mated together in an attempt to produce homozygous crested offspring. When the crested offspring were mature, they were tested by mating them to normal finches. All the young would be crested if the crested parent was homozygous. If one or more normal-headed young were raised, the crested parent was heterozygous.

If crested is a sex-linked dominant mutant, mating a crested female to a non-crested male would produce only crested male and non-crested female offspring. The numbers in Table 1 are the results of the putative sex-linkage of crested, which are low compared to the testcrosses and F_2-equivalent offspring. Most of the offspring were sold or otherwise donated before the sex was assuredly evident. Nevertheless, the numbers are sufficient to class crested as an autosomal mutant.

Table 1: Test for Putative Sex-Linkage of Crested, Crested Offspring from Eight Matings of Male Non-Crested, $Z^+//Z^+$ × Female Crested, $Z^{Cr}//W$

Male Crested, $Z^{Cr}//Z^+$	Male Non-Crested, $Z^+//Z^+$	Female Crested, $Z^{Cr}//W$	Female Non-Crested, $Z^+//W$	Total
14	26	13	26	**79**

Results

Sex-linkage is excluded by the data in Table 1. Note the deficiency of crested offspring in Table 1, 27 crested to 52 normal. This deficiency is repeated in F_2-equivalent offspring (Table 2) and birds from testcrosses (Tables 3 and 4).

Table 2: Crosses from Crested Males to Crested Females

Crested, Cr//Cr and Cr//+	Normal Non-Crested, +//+	Total
Observed (o) = 110	70	**180**
Expected (e) = 135	45	**180**
Deviation (d) = 25	25	–
d^2 = 625	625	–
$\Sigma d^2/e$ = 4.63	+13.89	**18.52 (χ^2)**
df = 1	$P \sim 0.0001$	–

Note: Offspring from 19 matings with the expected ratio of 3:1 of crested to normal offspring.

The probability of 0.0001 is highly significant. From the Chi-square results, only one time in 10,000 would expected results be that far off, if the hypothesis of a regular dominant is correct. Not only is a 3:1 ratio unacceptable, but even a 2:1 ratio has less than the expected numbers of crested offspring, leading to a $P = 0.14$ from a Chi-square test. The 2:1 ratio is expected from a hypothesis of a codominant lethal. That is, Cr//Cr do not survive to be counted. And the low number of crested leads to an idea that perhaps even the heterozygote, Cr//+, sometimes might not be viable.

Testcrosses information is given in Tables 3 and 4. The results are significant from the Chi-square results (Table 3). Less than three times in a thousand would observed results be that different from the expected results be that far off, if the hypothesis of a 1:1 ratio is correct. As with the F_2-like results, it is the crested class with deficient numbers.

Table 3: Cross of Crested Males with Normal Females		
Crested, Cr//+	**Non-Crested (Normal), +//+**	**Total**
o = 73	115	**188**
e = 94	94	**188**
d = 21	21	–
d^2 = 441	441	–
$\Sigma\, d^2/e$ = 4.69	+4.59	**9.38 (χ^2)**
df = 1	$P \sim 0.0025$	–
Note: Offspring from 15 matings with the expected ratio of 1:1 crested to non-crested (normal).		

The results in Table 4 are on the verge of significance. Nevertheless, again it is the crested class with deficient numbers. Pooling the results of Tables 3 and 4 should point in the same direction (Table 5).

Table 4: Cross of Crested Females with Normal Males		
Crested, Cr//+	**Non-crested = normal, +//+**	**Total**
o = 116	146	**262**
e = 131	131	**262**
d = 15	15	
d^2 = 225	225	
$\Sigma d^2/e$ = 1.72	+1.72	**3.44**
df = 1	$P \sim 0.058$	
Note: Offspring from 16 matings with the expected ratio of 1:1 crested to non-crested (normal).		

The Chi-square test results in Table 5 are highly significant. Less than six times in ten thousand would expected results be that far off, if the hypothesis of a regular dominant gene controlling crested was correct.

Alternative Test

Another approach is to find, or fail to find, a homozygous crested. One may take crested offspring from crested parents to test. Such a crested offspring has a 1/3 chance of being

Table 5: Cross of Crested with Normal Mates		
Crested, Cr//+	Non-Crested (Normal, +//+)	Total
o = 189	261	**450**
e = 225	225	**450**
d = 36	36	–
d^2 = 1,296	1,296	–
$\Sigma d^2/e$ = 5.76	+5.76	**11.52**
df = 1	$P \sim 0.00058$	–
Note: Offspring from 31 matings with an expected ratio of 1:1 crested to non-crested (normal).		

homozygous, if the character is not lethal when homozygous. This putative homozygous crested offspring, when mated to a normal non-crested bird, should produce all crested offspring. When all offspring from such a testcross are crested, then it may be stated that the crested parent is homozygous. How many offspring would be considered enough to 'prove' homozygosity of the crested parent? Seven crested offspring in sequence and lack of any normal-headed offspring would constitute a $(1/2)^7 = 1/128$ chance in being wrong in the assumption of homozygosity of the parent. Twelve crested offspring sequentially would constitute a $(1/2)^{12} = 1/4096$ chance in being wrong. Since zebra finches normally have a clutch of about five eggs (4-7?), one can choose the latter number of 12 to feel more confident in declaring a bird homozygous for crested. Such a bird has never been reported.

We have tested 25 such possible homozygous crested zebra finches, and all had one or more normal-headed offspring. Thirteen males and eight females were in the authors' breeding cages. The others were in isolated cages held by the volunteers.

For the probability, we can use the 'any order sequence of events' which reduces to the end term of the expanded binomial of algebra $(p^s + q^t)^n$, where p is the expected probability or frequency of homozygous crested finches found, s is the number of homozygotes occurring; q is the probability or frequency of heterozygous finches found; and t is the number of such heterozygotes tested. So $1p^0 q^{25} = 1(1/3)^0 (2/3)^{25} = 0.000004$. That is, we have a less than four in 100,000 or one in 25,000 chance of being wrong in saying that crested in zebra finches is a codominant lethal.

Discussion

Wild species of birds may have crests as a normal part of their phenotype. And, of course, no reduction in viability is evident. North American birds with crests include the cardinal, waxwing, wood duck, pileated woodpecker and blue jay. Of course, the zebra finch whorl or twist of head feathers (or that in the budgerigar) is unlike any of the normal wild species' crest. Crest in pigeons and ringneck doves is a genetic recessive (Levi, 1957) and does not exhibit any reduction in penetrance, although variability in expression is evident. In the pigeons, it varies from the very large hood crest in jacobins to the small peak crest in turbits (Levi, 1957). We have been unable to find data showing that crested in canaries and ducks is lethal when homozygous. The cause has been said (as common knowledge by fanciers) to be brain hernia in the developing embryo.

Martin (1985) noted that crested in zebra finches is a dominant and that "birds with monozygous genes for it …are not viable." Blackwell (1988), in his book on zebra finches wrote as a knowledgeable fancier and states "…a double factor for crests is usually regarded as being lethal with other species which are normally uncrested". And later in his book, he wrote "…and in most species of birds, the crested form is usually dominant genetically".

A few other species have been investigated. The budgerigar, *Melopsittacus undulatus,* is one with some literature about crest. The most complete discussion we could find is that by Clarke (2004). Clark appropriately introduced the genetic terms Penetrance and Expressivity (PE) and developed his PE theory to improve on the Initiatory theory adapted by the Crested Budgerigar Club. In Budgerigars, crest is said to be an autosomal dominant with low penetrance and highly variable expressivity. Clarke completely avoided the terms homozygous and heterozygous. He replaced them with double factor and single factor, using the genetic symbols CrCr and Crcr, respectively, without labeling the wild type gene with the plus superscript (as cr^+), now preferred by geneticists (Onsman, 1992; and Miller and Hollander, 1995). Clarke indicated that CrCr (homozygotes) always showed crest, but Crcr (heterozygotes) had only 17% penetrance. His data were pooled, resulted from 1,411 progeny, but never showed any family data proving a bird to be homozygous. How this could be done is debatable.

Clarke indicated that the expressivity of the crest in budgerigars was polygenic, but he finally, in one sentence, admits environmental influences are possible after a few pages of discussion on genetic modifying possibilities.

Onsman (1992) used the symbols Cr and Cr^+ for the budgerigar genes and calls crested in budgerigars a subvital character. He excluded sex-linkage and cited numerous data showing 48% lethality in both heterozygotes and homozygotes (!) of the dominant character crested. Onsman also cited Goessler (1938) as showing expression of crested being 'due to' abnormal involvement of the bone of the skull. Brain hemispheres were larger in Cr//Cr homozygotes than in $Cr//Cr^+$ heterozygotes, which were larger than normal $Cr+//Cr^+$. This leads to behavioral abnormalities in 25% of the heterozygous and homozygous mutant birds.

Pigeons and ringneck doves have ordinary Mendelian recessive inheritance of crested without detrimental results. But our results with the crested zebra finch are more like the budgerigar, with heterozygote sub-viability (or reduced penetrance) and variable expressivity. However, unlike the budgerigar (presumably), no homozygote has been found.

The most likely hypothesis for the genetic control of crested in zebra finches would be that it is a codominant lethal with reduced hatchability of some heterozygotes, or perhaps some reduced penetrance. Where crested and normal-headed finches should be in equal numbers, crested is reduced to 80-85% (189/225 and 110/135) by fledging. There were very few losses after the young developed pin feathers.

One wonders if crested canaries and ducks also have a somewhat reduced viability or penetrance. Where is the similar data for these species?

Hopefully, some other finch fanciers will be stimulated to obtain similar data. The next species to be so tested, perhaps, should be the society finch, *Lonchura striata*. Will they also show codominant lethal inheritance, and perhaps even reduced viability or penetrance?

Conclusion

Analysis of the breeding records to test inheritance of crest in zebra finches has yielded rather surprising data. Not only crest is a codominant lethal, but it is detrimental before hatching or before fledging, and/or has reduced penetrance.

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