AN ABSTRACT OF THE THESIS OF

Wendy R. Colvin for the degree of Bachelor of Science in Bioresource Research presented on June 10, 2002.
Title: Genetic Selection for Avian Embryo Development at an Elevated Temperature.

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A genetic selection study to determine the effects on egg hatchability and subsequent chick performance of Japanese quail eggs incubated at a 38.9° C (102° F) dry bulb temperature (Selected, S) when compared to 37.8 C (100°F) (Control, C) was conducted for four consecutive generations. Eggs from a randomly mated population of Japanese quail were randomly allocated to the two treatment groups and incubated in dedicated Jamesway 252 units for the first 14 days then transferred to a common hatcher 36.9° C (98.5° F). Using family-based selection, the chicks that hatched from the two lines were subsequently used as breeders (25 pair matings/line) and the resulting eggs incubated at their respective temperatures. After four consecutive generations, percent egg fertility, percent early dead embryos (1-7days), and percent hatch of fertile eggs were numerically higher in line C vs. S. While total development time was reduced in line S vs. C by 24 hours, mean egg weight and body weight were higher ($p < 0.01$) and age to first egg was reduced in line S.

# Genetic Selection for Avian Embryo Development at an Elevated Temperature

by
Wendy R. Colvin

A THESIS

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I understand that my thesis will become part of the permanent collection of the Bioresource Research program. My signature below aurhtorizes release of my thesis to any reader upon request.

Wendy R. Colvin, Author

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## CONTRIBUTION OF AUTHORS

Drs. Savage and Hermes assisted with data collection. Dr. Savage assisted in the writing of the thesis and the abstract, as well as with the interpretation of the data.

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## DEDICATION

Dedicated to my husband, John, children, Christine, Daniel, Karen, and Marie, my parents, Alvin and Fern Jones, and my friends, Christine, Bonnie, and Bev Rose, without whose support and understanding I could not have accomplished this.

# GENETIC SELECTION FOR AVIAN EMBRYO DEVELOPMENT AT AN ELEVATED TEMPERATURE

## INTRODUCTION

During the month of November, 2001, the U.S. Department of Agriculture (USDA) reported the production of 1.04 billion hatching eggs, of which 977 million were broiler-type and 60.0 million were egg-type. On December 1, 2001, there were 634 million broiler-type and 29.0 million egg-type eggs in incubators, for a total of 663 million eggs (NASS, 2001). These figures provide an estimate of the economic consequence associated with hatching chicken eggs in the U.S. alone. Since the poultry industry is profit-driven, it is constantly in search of ways to improve, and even small improvements in hatchability can result in substantial economic gains (Proudfoot, 1969).

Development of the chicken egg into a chick requires 21 days. Not all eggs incubated, however, will hatch. If improvements in the biology of incubation could be achieved by improving hatchability and reducing development time, thereby increasing the efficiency of incubation, the benefits to the U.S. economy would be substantial.

One measure of efficiency is hatchability, which in this paper is defined as the percent of fertile eggs hatched – the number of chicks hatched divided by the number of fertile eggs set, times 100 (Proudfoot, 1969). Hatchability may be influenced by many factors of an environmental and/or genetic nature (Decuypere *et al.*, 2001). These include length of storage of the eggs before setting (Woodard and Morzenti, 1975; Mather and Laughlin, 1976), egg storage temperature, which

should be below physiological zero for the species (Wilson, 1991), relative humidity in the incubator (Hoffmann, 1988), age and nutrition of the breeder hen (Beer, 1969), fertility, which can be inherited to some extent, shell quality, weather conditions (North and Bell, 1990), strain or breed of bird (Hutt, 1969), oxygen and carbon dioxide levels in the incubator (Decuypere, et al., 2001), and incubation temperature, which is one of the most critical factors (Wilson, 1991). Many of these factors are interrelated. For instance, if the breeder flock is older, eggs should be collected and placed in storage more often, and humidity levels must be adjusted for higher or lower temperatures (Wilson, 1991).

Of the various physical factors, incubation temperature is one of the most critical (Insko, 1949; Wilson, 1991). Lower incubation temperature extends the development time (time to hatch), whereas elevated temperature shortens development time, but increases embryo mortality (Romanoff, 1936). Studies have shown that the optimum incubation temperature for optimum hatchability is about 37° C (98.6° F), and lowering the temperature by 2° C after the 16$^{th}$ day improves hatchability (Lundy, 1969). Because of this, it is common industry practice to transfer eggs to a separate, lower-temperature hatcher for the last few days of incubation (Romanoff, 1936). The optimum temperature for early hatching, however is between 37.5 to 39 ° C (99.5-102.2° F). It has also been shown by Henderson (as reported in Lundy, 1969) that by beginning the incubation period at a higher temperature (40° C) and later reducing the temperature to 37.8° C, improved hatchability can be attained while accelerating the incubation period by one day (Lundy, 1969). Unless single-stage incubators are used, however, such a regimen might not be practical at the industrial level.

Typically, if the temperature of incubation is increased by as little as two degrees C, many embryos will die in the shell from hyperthermia, thus reducing hatchability (Lundy, 1969). In chickens, the influence of temperature on growth is greater during the earlier stages of incubation, ending at around the sixteenth day (Ande and Wilson, 1981). In 1937, Barrott (as reported by Tullett, 1995) concluded that the optimum incubation temperature was 37.8° C (100° F), with no more than a ± 0.3° C (0.54° F) variation. At 39.7° C (103.5° F), nearly all embryos die (Insko, 1949). However, those chicks that do hatch at an elevated temperature develop about 1.5 to two days earlier than those incubated at the normal temperature (Hoffmann, 1988).

The question is whether genetic selection of chicks successfully hatched at higher incubation temperatures can produce a bird better suited for this hatching environment without damaging commercially important traits such as hatchability, body weight, growth rate, or egg production (Smith et al., 1991). In this study Japanese quail (*Coturnix japonica*) were used as a biological model for the chicken. Japanese quail make an ideal pilot animal for poultry studies, as they have a rapid generation turnover, maturing at five to six weeks of age, thus allowing four or more generations per year (Wilson *et al.,*1961). By three weeks of age, their sexes can be easily determined, and they are extremely hardy and prolific (Padgett and Ivey, 1959). Japanese quail are also more economical than chickens, as they require less space and feed. In contrast, chickens mature in about 21 weeks, and only one generation per year is possible.

At the normal incubation temperature of 37.8° C, fresh quail eggs should hatch at no later than 17 days of incubation (Hoffmann, 1988), compared to the chicken, which hatches at 21 days of incubation.

Embryos surviving an elevated incubation temperature possess a unique genetic constitution. By repeatedly selecting for quail embryos with the ability to develop at a higher incubation temperature, there is a potential to produce a more genetically fit animal. A question that arises, however, is how other characteristics of this animal may be affected when genetic selection occurs, as altering incubation temperature is known to change metabolic rates in other species, with higher temperatures accelerating growth (North and Bell, 1990; Geers *et al.*, 1983).

If this experiment is successful with quail, it could theoretically be applied to other poultry species, as most genetic traits in one poultry species can be found in many of the others. Early hatching ability could have considerable economic benefits for the hatchery industry by shortening the incubation period, thus allowing more chicks to be hatched in the same amount of time, at lower energy costs.

# LITERATURE REVIEW

Besides physical factors, such as incubation temperature, humidity, turning, etc., there are genetic factors that influence hatchability (Landauer, 1961). Beaumont *et al.* (1997) computed heritabilities for various stages of embryonic death. They also determined a genetic correlation between fertility and hatchability, concluding that selecting for increased fertility should reduce embryonic death and vice versa. In an experiment in which eggs were incubated at 36.1, 37.6, and 39.2° C (97, 99.75 and 102.5° F), Byerly (1938) observed that eggs from certain hens tended to hatch well at all three temperatures, while those from others performed poorly at all three temperatures, which would indicate that there is a definite genetic component to hatchability that may be independent of incubation temperature. More recent studies also suggest that optimum incubation temperatures may vary between poultry strains (French, 1997). According to Decuypere *et al.* (2001), "There is a substantial endogenous or genetic component that contributes to the variability in the developmental parameters and may also interact with the environmental variables." Heritability estimates for reproductive traits in turkeys, including hatchability, have been estimated in several studies. Heritablitiy estimates for hatchability in the turkey have been calculated in several studies (McCartney, 1962), and range from relatively low (0.12) to high (0.919). Shook *et al.* (1971), also working with turkeys, were able to select against hatchability, decreasing it by 12% between the first two generations and 6% between the next two, by selecting for various stages of embryonic mortality.

# OBJECTIVES

The objectives of this study were to determine if genetic selection for the ability of quail embryos to develop at an increased incubation temperature can be realized, and to determine what effects this genetic selection may have on non-selected reproductive traits of economic significance. These traits include fertility, embryonic mortality at various stages (which influences hatchability), growth rates, age at onset of sexual maturity, and egg size.

# MATERIALS AND METHODS

## Incubation of Eggs — Base Population

Japanese quail eggs were obtained from a randomly mating flock maintained at Oregon State University. A total of 400 eggs were randomly assigned to treatment groups, designated as Control and Selected. They were set in trays modified to hold the smaller quail eggs in two Jamesway 252 single stage incubators, which had been set at 37.8° C (100° F) for the Control and 38.9° C (102° F) for the high temperature treatment groups (Selected), respectively. These incubators were equipped with automatic egg turners. Relative humidity was maintained at wet-bulb temperatures of 30° C (86° F) in the Control incubator and 31.1°C (88° F) in the Selected incubator (Landauer, 1961). On the fourteenth day of incubation, the eggs were removed from the incubators and candled to detect infertile eggs and early dead embryos. These eggs were broken out and examined to determine fertility and/or age at and cause of embryonic death (Padgett and Ivey, 1960). Since it is standard practice to lower incubation temperature for the last two or three days prior to hatching (Romanoff, 1936), those eggs which appeared to be developing normally (Controls and Selected) upon candling were placed in pedigree hatching baskets and set in a third Jamesway 252 incubator, which was set up as a hatcher at 36.9° C (98.5° F), with a wet-bulb temperature of 30° C (86° F). At this time, the eggs were no longer turned. Daily measurements of these temperatures were recorded by the farm staff on provided set sheets. Eggs were set weekly for four weeks to ensure adequate numbers of birds for breeding and to provide additional hatchability and embryonic mortality data. On the fifteenth day of incubation eggs in both treatment groups were examined to determine if pipping

had begun. When the majority of chicks within a treatment group had hatched, they were removed from the hatcher and wing-banded for identification. The chicks were then transported to Harrison Farm where they were randomly placed in electrically heated battery brooder units, with the Control and Selected birds intermingled to eliminate confounding environmental variables. Chicks were fed a commercial game bird starter diet (Purina® Game Bird Startena®). The farm staff was responsible for temperature management of the brooder units by decreasing the temperature by 2.8° C (5° F) per week according to the following schedule:

| | |
|---|---|
| Day 1 to 1 week | 35° C (95° F) at the back of the brooding unit |
| 1-2 weeks | 32° C (90° F) at the back of the brooding unit |
| 2-3 weeks | 29.4° C (85° F) at the back of the brooding unit |
| 3-4 weeks | 26.7° C (80° F) at the back of the brooding unit |
| 4-5 weeks | 23.9° C (75° F) at the back of the brooding unit |
| 5+ weeks | 18.3-23.9° C (65-75° F) |

At four weeks of age, the birds were transferred to colony growing cages and kept at ambient room temperature. Mortality records were kept (date, wing-band number, and cause of death, if known).

**Selection of Breeding Pairs**

At four weeks of age, the birds were weighed, beak-trimmed, and assigned to breeding pairs within their treatment groups (25 pairs per treatment). The control line consisted of randomly selected male and female quail. Several alternates were kept in case of mortality. Since their parentage was unknown, inbreeding may have occurred in this generation, but was avoided in successive generations.

The selected line consisted of quail that were hatched from eggs incubated at 38.9° C. Selection of breeder candidates was based upon the mean hatchability for each family. Only individuals whose family mean hatchability was equal to or

greater than the overall mean hatchability for the line were selected as breeders. The pair matings were established with avoidance of full sibling matings to limit inbreeding. Males of both treatment groups were kept separately from females for two weeks after onset of lay, in order to provide "virgin" eggs for examination for parthenogenesis. About a week after the males were introduced to the females, the birds were beak-trimmed in order to prevent further injuries/mortalities due to aggression. Breeding pairs were randomly assigned to 50 cages, with the odd-numbered cages holding the Selected birds and the even-numbered cages holding the Controls.

**Collection and Marking of Eggs**

When the hens were in full production, eggs were collected daily and marked with their corresponding pen numbers. Eggs were stored at 12.8-15.6° C (55-60° F) for no more than seven days (Woodard and Morzenti, 1975).

**Incubation—Generations Two, Three, and Four**

Eggs were sorted by cage number and placed in incubation racks for four weekly settings. They were incubated at the same temperatures and under the same conditions as for generation one, with the eggs from the control line being placed at 37.8° C. and those from the Selected line at 38.9° C. All other procedures were carried out the same as for the first generation, for a total of four generations, as of June 1, 2002 (at this time, the fourth generation birds are 8 weeks of age and a few have just begun to lay).

**Statistical Analysis**

All data were entered into a computer database (Excel). Two-sided t-tests were conducted to compare differences in body weights, egg weights, and age at onset of sexual maturity in the females (onset of lay) to determine differences between lines. Statistical tests were performed using the $S^+$ statistical software package.

# RESULTS

## Incubation Results

The influence of incubation temperature on fertility, incidence of early deaths (D1), late deaths (D3), pipped eggs, hatch of fertile eggs, and hours to hatch for generation 3 are summarized in Figures 1 to 6, respectively.

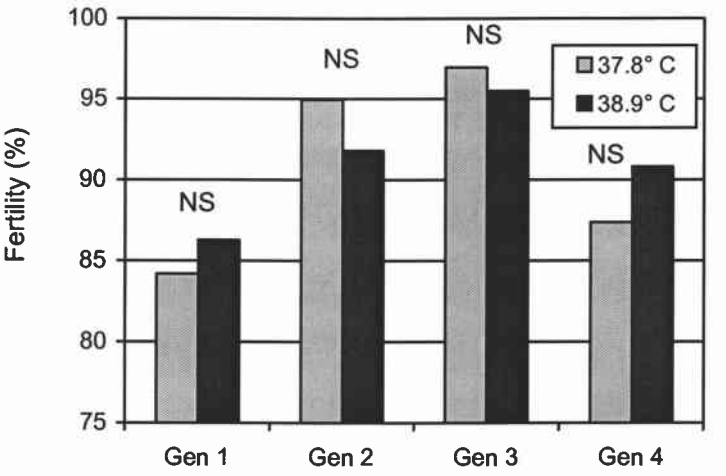


**FIGURE 1. Influence of Incubation Temperature on Egg Fertility**

Figure 1 summarizes the egg fertility results observed for the four consecutive generations studied. Upon performing two-sided t-tests, there were no significant differences between treatment groups in any of the four generation. The lower fertility in the first generation is possibly due to the advanced age of the farm's breeding population (Woodard and Abplanalp, 1967).

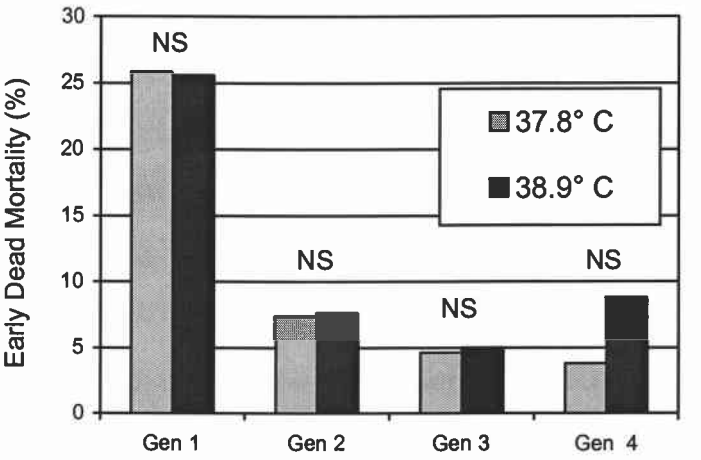


**FIGURE 2. Influence of Incubation Temperature on Early (1-7 days) Dead**

Figure 2 summarizes the observations recorded regarding the incidence of early dead (D1) embryos in the two treatment groups over the first four generations. The increased mortality at this stage in the first generation may be due in part to the age of the breeder population (Woodard and Albplanalp, 1967). There was no significant difference between the two treatment groups during the first three generations. In Generation 4, however, the incidence of D1s in the Selected group was significantly higher. It would be premature at this time to determine if this was due to the high temperature treatment or only due to chance (data from several more generations are needed). Statistical analyses were performed using two-sided t-tests.

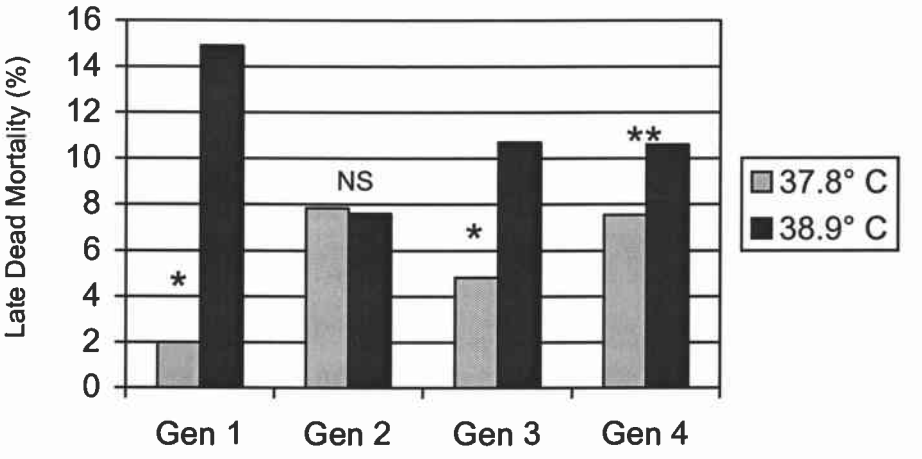


**FIGURE 3. Influence of Incubation Temperature on Late (14-18 days) Dead Embryos**

*$p<0.05$

**$p<0.10$

Figure 3 summarizes the incidence of late dead (D3) embryos over the first four generations. There was a significant difference between the Control and Selected embryos in the first generation ($p<0.05$). This was not unexpected, however, as high temperature incubation is known to increase embryo mortality during this period (Romanoff, 1972). This difference was also apparent in Generations 3 and 4 ($p<0.05$ and $p<0.10$ respectively). In Generation 2 there was no significant difference. It appears that selection for survival of high temperature incubation may slowly be decreasing the difference between the Control and Selected birds at this stage of incubation, but again, it is too early to be sure. Statistical analysis was performed using two-sided t-tests.

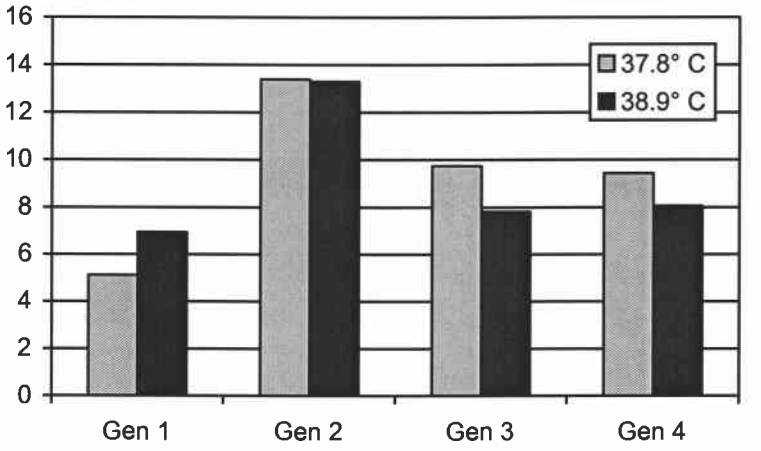


**FIGURE 4. Effect of Incubation Temperature on Pipped Embryos**

Figure 4 summarizes the percentage of pipped embryos observed in each generation (pipped embryos are those which begin hatching but are unable to finish). There were no significant differences between treatment groups in any of the four generations ($p>0.05$). Statistical analyses were performed using two-sided t-tests.

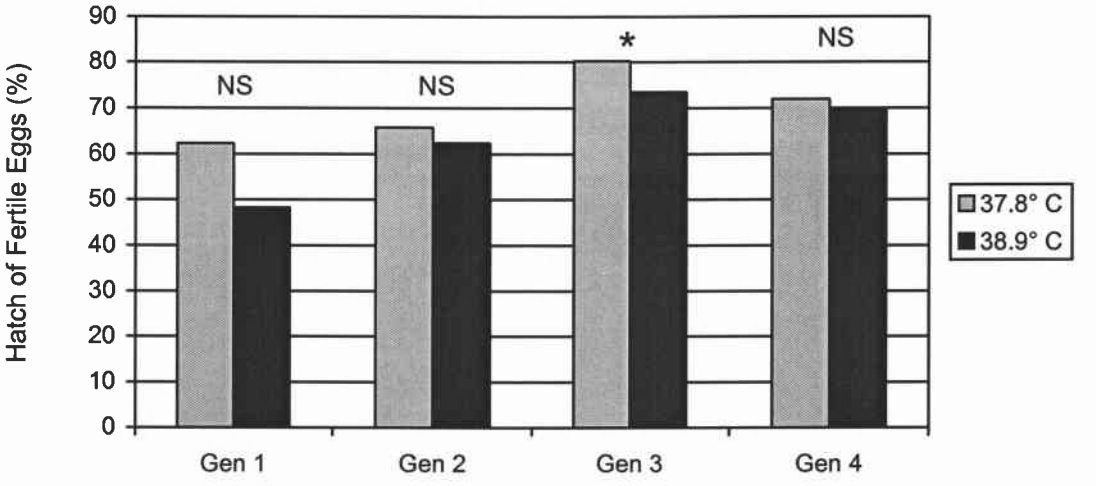


**FIGURE 5. Influence of Incubation Temperature on Hatch of Fertile Eggs**

*$p<0.05$

Figure 5 summarizes the percent hatch of fertile eggs for each respective generation. There was no significant difference between the Control and Selected treatments in the first, second, and fourth generations. In the third generation there was a significant difference ($p<0.05$), using a two-sided t-test. It is possible for a significant difference to occur by chance (Landauer, 1961), however, it does appear that what differences there are between treatments may be lessening.

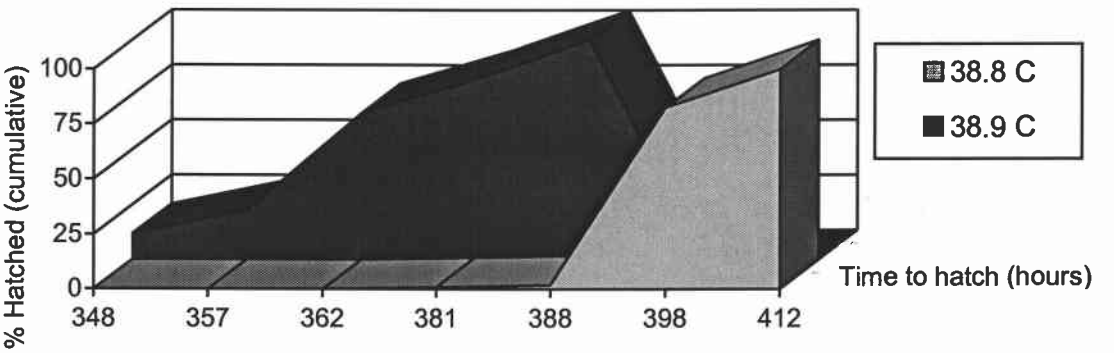


**FIGURE 6. Influence of Incubation Temperature on Time of Hatch for Generation 3**

Figure 6 provides an illustration of the relative times at which the chicks from the two treatment groups had developed in one hatch during the third generation, expressed as a cumulative percentage of the total chicks hatched per treatment group. The x-axis indicates the number of hours from the time of setting the eggs in the incubators until the chicks had fully emerged from their shells. The first Control chick did not hatch until 381 hours. The Selected chicks were pulled from the hatching incubator at 388 hours, and the Controls were pulled at 412 hours, exactly 24 hours apart. Similar results were observed in all four generations.

**Traits of Economic Interest**

Body weights (male and female), age at onset of sexual maturity, and egg weights, are summarized in Figures 7 to 10, respectively. Body weights were compared separately by sex due to sexual dimorphism for this trait (Etches, 1996).

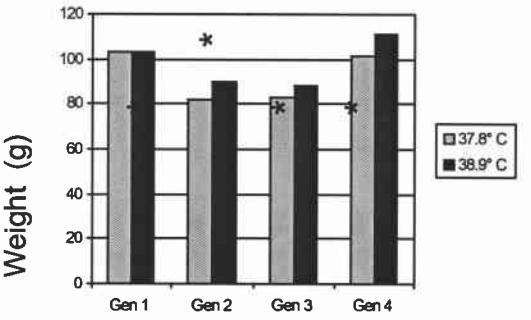


**FIGURE 7. Influence of Incubation Temperature on Female Body Weight**
*p<0.05

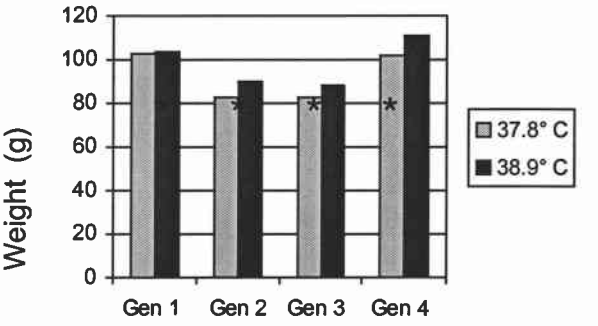


**FIGURE 8. Influence of Incubation Temperature on Male Body Weight**
*p<0.05

Figures 7 and 8 illustrate the mean body weight of the females and males of each generation respectively. Two-sided t-tests were performed, indicating that significant differences existed between the two lines for both sexes in each generation ($p<0.01$), with the Selected birds being heavier than the Controls. The birds were weighed at five weeks of age in the first and fourth generations and at four weeks of age in the second and third generations. One possible reason for the observed weight differences between lines could be that the Selected chicks were one day older than the Controls due to the elevated incubation temperature. The question remains whether a one-day difference in age can totally account for the observed weight difference (perhaps this will be answered in future generations). Other studies have shown, however, that elevated incubation temperature can

have an effect on thyroid hormones (Kühn *et al.*, 1982), growth factors in turkeys (Nestor et al., 1972), and metabolic rate (Romanoff and Faber, 1933; Geers, *et al.*, 1983). This could be a possible explanation for the accelerated growth rate in the Selected quail.

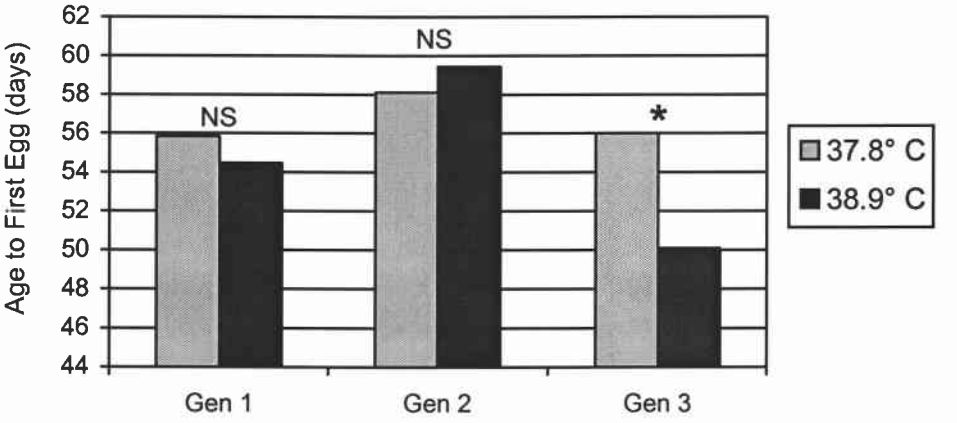


**FIGURE 9. Influence of Incubation Temperature on Age to First Egg**
*$p<0.001$

Figure 9 summarizes the results of high temperature incubation on the age to the first egg, which in females is the onset of sexual maturity. Two-sided t-tests were conducted, which determined that there were no significant differences between treatment lines in the first two generations. In the third generation, however, the difference is significant ($p < 0.001$). Again, this could be due to an increased metabolic rate in response to high-temperature incubation.

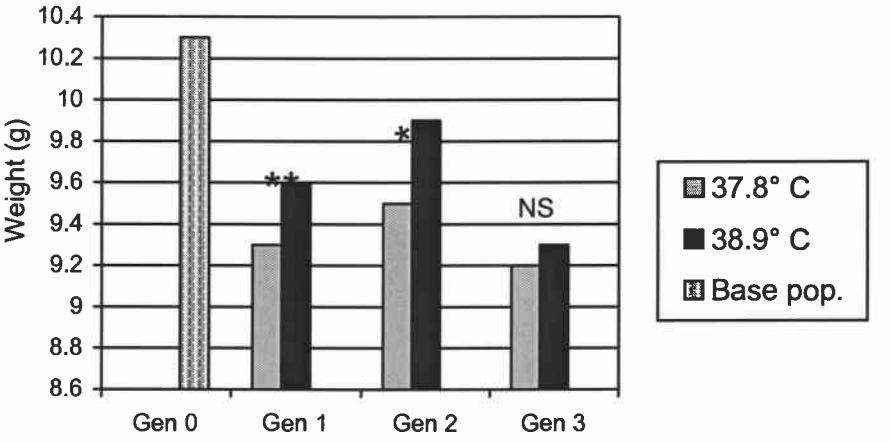


**FIGURE 10. Influence of Incubation Temperature on Egg Weight**

*$p<0.05$

**$p<0.10$

Figure 10 summarizes the data obtained for each generation's egg weights. Generation 0 (the base population) is represented by a single bar on the graph, as the eggs from both treatment groups came from a common pool. It is probable that these eggs were larger than those of the subsequent generations due to the age of the breeders (Woodard and Albplanalp, 1967). There were significant differences in egg weights between treatment groups in Generations 1 and 2 ($p<0.10$ and $p<0.05$ respectively), with the Selected eggs being heavier than the Controls. In the third generation the difference between the two treatment groups was not significant. It is possible that the difference in egg weights may be due to a difference in metabolic rates due to increased incubation temperature, but it is premature to make any conclusions at this time.

# CONCLUSIONS

From this selection study the results indicate that selection for the ability to survive increased incubation temperature is possible in the Japanese quail. The higher incubation temperature does serve to shorten the length of time required for embryonic development, but there are consequences to the embryo and its ultimate adult performance. A concern, however, is that with only three generations of selection, it is premature to formulate long-term conclusions.

As this is an ongoing project (projected to continue for at least 12 generations total), several further areas of study are suggested by these results. It would be advisable either to weigh the Selected and Control birds on consecutive days or to set them in the incubator a day apart, so that their ages are the same at the time they are weighed. This would remove a possible source of bias from the results. Another area of interest would be the length of the laying period (longevity) – since the Selected birds appear to be growing faster, would they also cease production or die at an earlier age? Physiological studies could also be performed, such as assays for growth factors or thyroid hormones, in order to determine why the Selected birds appear to be growing and maturing faster. Also, at some point in time, reciprocal settings of the eggs from each treatment group could be done, so that eggs from each line are incubated at each temperature.

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