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A genetic selection study to determine the effects on egg hatchability and subsequent chick performance of Japanese quail (*Coturnix japonica*) eggs incubated at 100 F dry bulb temperature (Control, Line C) when compared to other eggs incubated at 102 F (Selected, Line S) was conducted over 10 consecutive generations.

Eggs from a randomly mated population (designated as Generation 0) of Japanese quail maintained at the Oregon Agricultural Experiment Station were randomly allocated to two treatment groups (Lines C and S) and incubated at the different temperatures in separate but identical Jamesway 252 machines. On day 14 of incubation all eggs were transferred to a common hatcher (98.5 F). Using family-based selection, the chicks that hatched from the two lines were subsequently used as breeders (25 paired matings per line) and the resulting eggs from each line incubated at their respective temperatures for 10 consecutive generations. Following the 10th generation percent egg fertility and percent hatch of fertile

eggs were greater in Line C vs. Line S ($p<0.03$ and $p<0.0001$, respectively). Embryo development time was shortened in Line S by 24 hours and mean 4- or 5-week body weights were greater ($p<0.001$) in Line S. Ten-day post-hatch mortality increased greatly in Line S vs. Line C after generation 6 ($p<0.001$) and hen-day egg production decreased after generation 4 in Line S vs. Line C ($p<0.0001$).

The results indicate that embryo development time can be reduced by high temperature incubation, but at the expense of reproductive traits such as egg production, fertility, and hatchability of fertile eggs.

Selection for Hatchability of Japanese Quail Embryos Incubated at 102 F

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request

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Wendy R. Colvin, Author

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (Continued)

DEDICATION

This thesis is dedicated to my husband, John, my children: Christine, Daniel, Karen, and Marie, my parents, Alvin and Fern Jones, and my mother-in-law, June Rose, without whose patience, support, and prayers this project could not have been completed.

SELECTION FOR HATCHABILITIY OF JAPANESE QUAIL EMBRYOS INCUBATED AT 102 F

Introduction

During the month of December 2004, the U.S. Department of Agriculture (USDA, NASS, 2005) reported the production of 1.08 billion hatching eggs, of which 1.02 billion were broiler-type and 62 million were egg-type. On January 1, 2005, there were 651 million broiler-type and 34.4 million egg-type eggs in incubators, for a total 685.4 million eggs (NASS, 2005). These figures provide an estimate of the economic importance 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 production, and even small improvements in hatchability can result in substantial economic gains (Proudfoot, 1969).

Transformation of the chicken egg into a chick requires 21 days, although not all eggs incubated will hatch. If the efficiency of incubation could be increased by affecting improvements in the biology of incubation, thereby improving hatchability while concurrently reducing development time, the benefits to the U.S. poultry industry could be substantial.

All biological processes, including embryonic development, are a combination of genetic and environmental factors (Moore, 2001). In vertebrate embryos, tissues and organ systems develop as a result of cascades of inductive reactions that initiate the developmental program for each particular tissue or organ. Deviations

from these pathways are controlled genetically and developmentally by threshold reactions (Hall, 1999). Environmental stress can be utilized to destabilize a developmental system, thus revealing previously concealed genetic variation (Gilbert, 2003). It should be noted that genetic selection can only edit variations already in existence within the population—one cannot create new alleles by this method (Campbell, 1996). Since environmental responses are genotype-dependent and the capacity for an individual to respond to its environment within its lifetime is heritable (Hall, 1999), it is theoretically possible to alter embryonic development traits by genetic selection, environmental manipulation, or a combination thereof. One such environmental stress could be elevated incubation temperature, which is known to affect both hatchability and development time (Barott, 1937). In such cases, where the phenomenon of genotype-environment interactions exist, it may be useful to selectively breed for further improvement within a breed or line that is suitable for a particular environment (Mathur, 2003).

Genetic assimilation is "the process by which a phenotypic character initially produced only in response to some environmental influence becomes, through a process of selection, taken over by the genotype, so that it is formed even in the absence of the environmental influence that at first had been necessary" (King and Stansfield, 1997). The genetic assimilation of a phenotypic modification resulting from an environmental stress may eventually be attained by artificial selection (Waddington, 1962). Through genetic assimilation, phenotypic change is related to

genotype and environment in terms of specific environmental stimuli, epigenetic interactions, and canalization of development. (Hall, 1999).

In wild-type populations, many traits appear to be almost invariant (Waddington, 1962). This phenomenon is ascribed to canalization, "the existence of developmental pathways that lead to a standard phenotype in spite of genetic or environmental disturbances (King and Stansfield, 1997). In other words, development appears to be "buffered" so that slight genotypic mutations or environmental changes fail to effect phenotypic changes. Therefore, certain developmental outcomes are more likely to occur than others, regardless of minor variations in the genome or the environment (Moore, 2001).

When a trait is highly canalized, it may develop in many different environments, and therefore appear to develop independently of environmental factors. On the other hand, traits which are less canalized tend to be much more sensitive to an organism's developmental environment. A trait, however, can be influenced by environmental manipulations regardless of how highly canalized its development (Moore, 2001), thus allowing the accumulation of unexpressed recessive mutant alleles in a genome (Lerner, 1954). One such environmental challenge is incubation temperature, for which there is a specific range under which normal embryonic development is seen, with deviations above or below this range resulting in embryonic failure in most individuals (Lundy, 1969). Hypothetically, individuals capable of hatching at temperatures outside the normal range may

possess a unique genetic makeup that could be manipulated by artificial selection for traits such as hatchability.

A related process, genetic homeostasis, defined by Lerner (1954) as "the property of the population to equilibrate its genetic composition and to resist sudden changes," can lead to problems when artificial selection is employed to enhance a particular trait within a population, since natural selection favors intermediate rather than extreme phenotypes (Lerner, 1954). "The best adapted form in a species is usually one that is close to the average in all quantitatively varying characters" (Wright, 1951, as quoted in Lerner, 1954). In other words, there is an optimum range for traits such as body weight, egg weight, etc., and individuals falling too far in either direction from this optimum tend to exhibit reduced reproductive fitness (*e.g.*, reduced hatchability in poultry species). In broiler breeders, for example, artificial selection for increased broiler growth has led to a divergence from the optimum body weight for successful reproduction, resulting in disorders such as infertility, as well as defective ova, spermatozoa, and embryos. This may be due to "neuroendrocrine imbalances, disrupted synchrony in gametogenesis, dysfunctions in ovulation-oviposition patterns, and reduced libido" (Siegel and Dunnington, 1985). Maximum hatchability in turkeys and chickens occurs when eggs of intermediate size are used (Lerner, 1951), and selection for increased egg size has been shown to reduce reproductive fitness (Lerner, 1954).

A potential problem in any artificial selection experiment is correlated response to selection, the "genetic change in one or more traits resulting from selection for another" (Bourdon, 2000). Possible genetic mechanisms for this phenomenon include linkage, "the occurrence of two or more loci of interest on the same chromosome" (Bourdon, 2000), and pleiotropy, "the phenomenon of a single gene affecting one or more trait" (Bourdon, 2000). Since linkage is sooner or later disrupted by chromosome recombination due to crossing over during the meiotic process, it is generally only a temporary cause of correlated response to selection—the effects are likely to only last for a few generations at most. Pleiotropy is the major cause of correlated response. Related polygenic traits are most likely influenced by multiple genes with pleiotropic effects (Bourdon, 2000). In addition, Decuypere *et al.* (2003) suggest that some genetic correlations may be due to "shared physiological control systems." Lerner (1951) suggests that overdominance, epistasis, and balanced heterozygosity of chromosomes or whole chromosomes may also be involved in genetic homeostasis. There is generally a penalty to be paid for the gains achieved from selection in an extreme direction for a given trait. This loss is generally in the area of reproductive fitness (Lerner, 1954). For this reason, selective breeding to enhance one trait may lead to adverse effects on other traits. For instance, Nestor and Noble (1995) found that selection for increased egg production in turkeys decreased body weight and egg weight, while selection for increased mature body weight adversely affected fertility and the hatch of fertile eggs while increasing egg weight. Selection for increased body weight in turkeys also is associated with decreased production of normal eggs

(Nestor, 1985). Likewise, selection for increased shank length in broilers can decrease hatchability (Lerner and Dempster, 1951). Similar results have been observed in waterfowl, where there is an antagonistic relationship between rapid growth rate and high reproductive performance (Bednarczyk and Rosinski, 1999), while in domestic poultry there is a strong negative relationship between increased body weight and reproductive ability, especially in meat-type parent stocks (Decuypere *et al.,* 2003). Hypothetically, artificial selection to improve incubation traits such as hatchability could potentially have similar consequences, in which correlated response might alter other traits of interest to the poultry industry such as egg production, body weight, etc.

There is a potential economic benefit to reducing development time without adversely affecting hatchability. The objective of this study was to determine if it is possible to develop a genetic strain of poultry that is capable of hatching at an elevated incubation temperature without sacrificing hatchability. It is hypothesized that if there is a genetic component to an embryo's ability to hatch at a higher incubation temperature, then selecting for this trait should lead to a genetic line with improved hatchability at that temperature.

Literature Review

Normal Requirements for Successful Artificial Incubation

"From the moment of fertilization of the ovum and the initiation of embryogenesis, embryonic development is affected by its environment" (Wilson, 1991). The formation of an embryo is the result of "interacting and self-regulating systems with differentiation and induction being the most conspicuous phenomena" (Al-Thani and Simkiss, 1992). Vick *et al.* (1993) describe the hatching process as "a delicate equilibrium among several factors," including inherited physical characteristics of the hatching egg, as well as external environmental factors. "During incubation a complex of biological processes, operating in parallel and in series with each other, is influenced by the action and interaction of the many stimuli" (Lundy, 1969). These environmental conditions include: oviductal time and unspecified oviductal conditions, post-oviposition pre-incubation holding environment and length of storage, and incubational environment, including temperature, humidity, gaseous environment, egg turning and egg orientation. Even under ideal conditions, not all eggs set in the incubator successfully hatch. "Failure of eggs to hatch is determined by two factors: infertility and embryonic mortality prior to and during incubation" (Kuurman *et al.*, 2003). In chickens, embryonic mortality occurs most often during the first week of incubation, least often during the second week, and at an intermediate level during the third week (Kuurman, *et al.,* 2001). These peaks of mortality tend to occur at critical stages

during which the physiological and developmental functioning of the embryo change at relatively high rates, such as during vascular formation. Japanese quail likewise exhibit two peaks of embryonic mortality, during the first three days of incubation and just prior to hatching; there is also a slight mid-incubation peak in mortality at around 10-13 days of incubation (Woodard *et al.*, 1973). Avian embryonic mortality can be attributed to many factors, genetic or environmental, such as chromosomal abnormalities or the inability of the eggshell to sufficiently exchange water and respiratory gases (Kuurman *et al.*, 2001; Romanoff, 1934), which affect physiological and developmental functioning of the embryo (Kuurman *et al.*, 2003).

Fertility

Since infertile eggs do not hatch (with parthenogenesis being a notable exception, Olsen, 1965), fertility is of high importance to the hatching egg industry. Fertility is calculated as the number of fertile eggs divided by the total number of eggs set (Yoo and Wientjes, 1991). The most common measure of fertility is the proportion of eggs in which embryonic development is or was proceeding, which is ascertained by light candling of the eggs. "Candling fertility" is an underestimate of fertility, however, as early dead embryos appear to be infertile if "clear" eggs are not examined after candling (Etches, 2000). Fertility can also be determined by microscopic examination of the egg yolk to determine the quantity of sperm attached to the vitelline membrane (Etches, 2000).

Fertility (or lack thereof) in broilers is greatly influenced by laying house management (North and Bell, 1990). Broilers on an *ad libitum* diet are often overfed, which can lead to a reduced number of sperm reaching the area of fertilization, although it is unclear whether this is a problem of poor sperm quality or due to a hostile environment in the oviduct (Renema *et al., 2003*). Infertility, which is the failure of the male and female gametes to unite, is usually caused by a failure to mate or by the deposition of poor-quality semen in the female reproductive tract. Failure to mate is generally caused by either an insufficient number of males in the breeder flock or obesity in the male (Etches, 2000). According to Funk and Irwin (1955), other factors which may affect fertility include sperm motility, proper breeder nutrition (male and female), endocrinology, the male's exposure to light, breeder age (male and female), rate of egg production (females producing eggs at high rates tend to produce eggs with higher fertility than those laying fewer eggs), preferential mating (if a hen is laying infertile eggs, breeding her to a different male may increase fertility substantially), seasonality (fertility tends to be high during the spring and low during the summer), and inheritance. Bernier *et al.* (1951) postulated that complete infertility could also be due to oviductal conditions preventing ascension of spermatozoa to the infundibulum or preventing their survival. Infertility could also be due to an irregular egg composition preventing fertilization from occurring. In Japanese quail, infertility may be due to improper male:female ratio, breeder flock age (especially in males), and improper management, *i.e.*, insufficient feeding and drinking space, overcrowding, an improper lighting regime, and undue stress

(Shanaway, 1994). In 1937, Byerly and Godfrey (as cited by Parker and Bernier, 1950) reported a linear relationship between fertility and female to male ratio, with fertility decreasing as the ratio of females to males increased.

In a breeding flock of New Hampshires, it was reported that a minimum of six to seven males per hundred females was required for consistent high fertility (Parker and Bernier, 1950). Breeding for increased egg production may also increase fertility, and inbreeding often reduces fertility in turkeys and chickens (Funk and Irwin, 1955). In Japanese quail the highest rate of fertility is obtained with a mating ratio of one male to two or fewer females (Woodard and Abplanalp, 1967). The first fertile egg is laid on the second day after introduction of males to females, and the duration of fertility is about nine to ten days after removal of the males (Woodard and Abplanalp, 1967). Very young quail cocks sometimes exhibit reduced fertility, and maximum fertility occurs between 12 and 15 weeks of age (Shanaway, 1994). In Japanese quail, Wilson *et al.* (1961) observed that hatchability and fertility declined markedly after about 24 weeks of age, with a concomitant increase in embryonic mortality during the mid-incubation period.

Fertility is, to some extent, an inherited factor, and is therefore a trait that can be improved over several years of continuous selection (North and Bell, 1990). Froman *et al.* (1999) observed that fertility was a function of sperm mobility, which they defined as "the net movement of a sperm population." For this reason, sperm mobility is a primary determinant of fertility. It has been demonstrated that sperm mobility can be heritable, with both sires and dams exerting an important

genetic influence on the trait (Froman *et al.,* 2002). Certain mutations, such as the "R" allele for rose comb, are known to be correlated with infertility (North and Bell, 1990). In quail, several fertility-related traits are inherited, including semen production, motility and concentration of spermatozoa, and semen volume (Shanaway, 1994).

In chickens, turkeys and quail, the last eggs to be fertilized may exhibit characteristically abnormal embryo development, while in Japanese quail, decreased hatchability has been attributed to ageing effects of sperm (Sittmann and Abplanalp, 1965). Duration of fertility, which is defined as "the interval between insemination and oviposition of the last fertile egg" (Etches, 2000), and is normally five to seven days, can be slightly shortened by inbreeding (Sittmann and Abplanalp, 1965). Inbreeding has been observed by many researchers to adversely affect fertility. Inbreeding does not directly affect fertility—but indirectly—possibly by altering egg composition, oviductal anatomy, or female sexual behaviors such as receptivity (Bernier *et al.,* 1951). In Japanese quail, fertility is strongly reduced under inbreeding (Sittmann and Abplanalp, 1965), and *Coturnix* exhibit a pronounced sensitivity to inbreeding, almost twice as severe as in chickens or turkeys, with adverse effects on hatchability, viability, and egg production (Woodard *et al.*, 1973).

Hatchability

Hatchability, a measure of incubation success, is usually expressed either as the percentage of chicks hatched from the total eggs set in the incubator or the

percentage of chicks hatched from the number of fertile eggs set (Lundy 1969; Proudfoot, 1969). Generally, "hatchability" denotes the percentage of fertile eggs that hatch and produce viable chicks (Landauer, 1961; Etches, 2000). Since fertility cannot be ascertained simply by candling, those eggs that appear "clear" upon candling must be broken out and examined to determine true infertility as opposed to early embryonic mortality (North and Bell, 1990).

Embryonic mortality, which is 100% of fertile eggs minus the percent hatchability, is often subdivided according to the stage of incubation at which death occurred (Etches, 2000). Scientists classify these stages differently. For instance, early embryonic mortality (in chickens) is calculated as the number of dead embryos up to day seven of incubation divided by the number of fertile eggs set (Yoo and Wientjes, 1991). North and Bell (1990) classify embryonic mortality as Period I (Preovipositional mortality), Period II (Early-dead embryos), Period III (8- to 18-day mortality), and Period IV (19^{th}, 20^{th}, and 21^{st} days of mortality). In Japanese quail (*Coturnix japonica*), embryonic mortality can be classified as D1 (during the first 7 days of incubation), D2 (days 8-14), D3, also known as "dead in shell" (days 15-17), and pipped, which is the method utilized by this author.

Factors Affecting Hatchability

There are many factors, both environmental and genetic, which affect hatchability in avian species. These include breeder age and nutrition, egg storage conditions, egg position and turning, incubator conditions, and genetics.

Breeder Age and Nutrition

The eggs of young pullets usually do not hatch as well as those from older breeder hens, due to reduced early embryonic viability (Crittenden and Bohren, 1962). Lowe and Garwood (1977) observed that embryonic growth rate was depressed in eggs from younger parents. This early embryonic mortality has been associated with increased eggshell membrane thickness in pullet eggs (Vick *et al.*, 1993). The first eggs laid by a breeder pullet generally are held within the oviduct for a longer than normal period, which may cause detrimental preincubation to occur. As hens age, however, their eggs become larger with thinner shells and are retained in the oviduct longer than during mid-lay (North and Bell, 1990). For both turkeys and chickens, hatchability tends to be highest during the first laying year (Funk and Irwin, 1955). Generally, hatchability increases from about the second until about the 12^{th} to 13^{th} week of lay, at which time it gradually decreases over time the longer the hen is in production (North and Bell, 1990).

Hatchability increases and length of the incubation period decreases as pullets age, due to changes in internal and eggshell quality, (Peebles *et al.*, 2001a). The physical nature of egg components changes as the breeder hen ages, affecting gas exchange between the embryo and the surrounding environment. Eggs with longer shell pores and thicker eggshells are produced by younger hens, while maximum hatchability is observed midway during the laying period when shell porosity is at its highest and shell thickness is at the lowest. Albumen quality is also known to decline with hen age. (Vick *et al.*, 1993; Brake *et al.*, 1997; Peebles *et al.*, 2001a).

Increased late embryonic mortality is often observed as the egg surface area to volume ratio changes as the breeder flock ages, due to an increase in egg weight exceeding the increase in surface area (Vick *et al.*, 1993). Eggshell quality can be impacted by breeder hen age (the shell generally thins with age), which then affects embryogenesis by impacting embryonic yolk uptake, growth, and body composition (Peebles *et al.*, 2001b). Young hens produce thicker-shelled eggs than do older hens, leading to imbalances in body moisture content in relation to total embryonic dry matter accumulation during the final week of incubation (Peebles *et al.*, 2001a).

In Japanese quail, aging of hens produces strong adverse effects on hatchability, whereas aging of males appears to have no effect. Young females tend to mate more frequently than older ones, regardless of the age of the male, but young males tend to successfully complete twice as many matings as old males (Wilson *et al.*, 1961; Woodard and Abplanalp, 1967).

Everything found in a hatching egg (except the sperm) is deposited there by the hen. Therefore, the hen's diet must contain adequate nutrients to provide the eggs with all nutrients necessary for the embryo to develop and hatch (Funk and Irwin, 1955). For normal embryonic growth and development to occur, the egg must contain a complete supply of all required nutrients. If the egg should contain inadequate, excessive, or imbalanced levels of nutrients, the results could be damaging or even lethal to the embryo (Wilson, 1997).

Genetic variation in the absorption, metabolism, and/or deposition of nutrients into the egg can affect nutrient effects on embryonic viability. It is possible that marginal nutrient deficiencies would only affect the most genetically susceptible breeder hens. Also, there can be genetic differences in nutrient requirements or metabolism. For example, clubbed down can result from genetic interference in riboflavin utilization (Wilson, 1997). One indicator of a nutritional problem is an increase in embryonic mortality from eight to 14 days of incubation, a period during which embryonic mortality is minimal (Tullett, 1990). Hatchability can also be affected by nutritional deficiencies resulting from damage to the breeder hen's intestinal tissues by parasitic organisms, even though the feed may contain adequate nutrients (Beer, 1969).

Many nutrients which can affect hatchability include vitamins, minerals, protein, fats, and fatty acids. Vitamins that influence hatchability include: vitamin A (retinol), biotin, riboflavin, vitamin B_{12}, pantothenic acid, thiamine, niacin, folic acid, pyridoxine, and vitamins K, D, and E (Jordan, 1990). Minerals that can influence hatchability include: selenium, iodine, manganese, calcium, phosphorus, magnesium, zinc, boron, chloride, potassium, and aluminum (Jordan, 1990). A low energy-high protein diet tends to produce eggs with reduced hatchability resulting from increased mortality in mid-incubation and unhatched pips (Wilson, 1997). Diets containing certain cyclopropenoid fatty acids (found in kapok seed meal and cottonseed meal) have been found to reduce hatchability to almost zero (Tullett, 1990). Specific vitamin deficiencies can also adversely affect hatchability, *e.g.*, a

deficiency of vitamin K can cause embryonic hemorrhage, while insufficient riboflavin can result in clubbed down, dwarfing, edema, micromelia, and degeneration of the myelin sheath of the peripheral nerves. Nutrient interactions can also occur, *e.g.*, vitamin B_{12} deficiency intensifies a pantothenic acid deficiency and vitamin D is essential for proper calcium metabolism (Beer, 1969). Numerous other nutritional deficiencies are known to affect hatchability (see Jordan, 1990; North and Bell, 1990; and Wilson, 1997 for more in depth discussions).

Pre-Incubation Egg Storage Conditions

Egg storage conditions such as temperature, length of time, and relative humidity play an important role in the success or failure of avian incubation. At the time of oviposition, chicken embryos are in the pre- or early gastrula stage (Wilson, 1991). For optimal hatchability, fertile eggs should be stored below physiological zero for chickens, which is the temperature below which the embryo remains in a state of suspended development and metabolism is minimal (Wilson, 1991). Physiological zero has been reported by various authors to be between 19 and 27 C (66.2 to 80.6 F; Brake *et al.*, 1997; Lundy, 1969), and between 21 and 23 C (69.8 to 73.4 F) for Japanese quail (Shanaway, 1994). Funk and Biellier (1944) found that embryonic development is reinitiated in chicken eggs at temperatures approaching 80 F (26.7 C), however, it would appear that various embryonic tissues may have differing physiological zeros, which may result in disproportionate development due to

uneven or unsynchronized growth when held too close to temperatures around 27 C (80.6 F; Wilson, 1991).

Optimum storage temperature may vary, depending upon the length of time the eggs are to be stored. Maximum hatchability is obtained when eggs stored for shorter periods are stored at higher temperatures. Mayes and Takeballi (1984 as, cited by Wilson, 1991) recommended that chicken eggs stored less than three days be held at 18 to 30 C (64.4 to 86 F), those held for three to seven days should be stored at 16-17 C (60.8 to 62.6 F), and those stored more than seven days should be held at 10 to 12 C (50 to 53.6 F). Card and Nesheim (1972) recommend 59 to 60 F (15 to 15.6 C) for eggs stored up to one week and 50 to 55 F (10 to 12.8 C) for eggs stored longer. Sainsbury (2000) recommends a storage temperature of 13 to 16 C (55 to 60 F.). Recommended storage temperature for Japanese quail eggs varies. Woodard, *et al.* (1973) recommend a temperature of 13 C (55.4 F), while Shanaway (1994) recommends a temperature of 18 C (64.4 F) when eggs are stored between one and three days, 16 C (60.8 F) for four to seven days, and 15 C (59 F) for greater than seven days.

Even under optimal storage conditions, hatchability declines (embryonic mortality increases) if eggs are stored for an extended period of time (Yoo and Wientjes, 1991; Kuurman *et al.*, 2002). Mather and Laughlin (1976) observed that hatchability of chicken eggs stored for 14 days was 19.5% lower than in unstored eggs. Eggs set the same day they are laid do not hatch as well as those that have been stored for one to four days (Funk and Irwin, 1955). Chicken eggs should not

be stored for more than one week (Brown, 1979), while a limit of four days is preferable, as there is almost no loss of hatchability during this period under proper storage conditions (Sainsbury, 2000).

Prolonged egg storage may result in an embryonic folic acid deficiency (Wilson, 1997). Fasenko and Robinson (2003) observed that as broiler egg storage increased, hatchability and embryo survival decreased, leading to the recommendation that eggs be stored for no longer than seven days. In turkeys, increased storage time leads to depressed embryonic metabolism and delayed growth. Exposing eggs stored for 15 days to an incubation temperature of 37.8 C (100 F) during the initial 14 days of incubation, however, can compensate and increase livability as compared to controls incubated at 37.5 C (99.5 F) (Christensen *et al.,* 2003a). These embryos also exhibited enhanced embryonic carbohydrate metabolism and elevated thyroid hormone concentrations (Christensen, *et al.*, 2003b). Although Sainsbury (2000) and Woodard *et al.* (1973) recommend storage of no longer than one week, quail eggs may be held as long as two weeks at 25 C (77 F) without substantially affecting viability (Padgett and Ivey, 1959). In *Coturnix*, pre-incubation embryonic mortality increases significantly after the third week of storage (Wilson *et al.*, 1961).

Relative humidity (RH) is another egg storage factor, which prevents evaporation and resulting enlargement of the air cell (Funk and Irwin, 1955). An RH of 75 to 80% for chicken eggs is recommended (Card and Neshiem, 1972; Sainsbury,

2000), and no less than 75% for quail (Sainsbury, 2000). Shanaway (1994) recommends for *Coturnix* that the RH should be between 75 and 80%.

Egg Position and Turning

Eggs should be positioned in the incubator with the large ends up, which allows the embryo to maintain the proper orientation within the egg. When eggs are set with the small end up, about 60% of the embryos develop malpositions, having their heads in the small end of the egg (North and Bell, 1990). Failure to turn eggs also increases malpositions and malformations. Malpositions are lethal because they prevent the embryo from attaining the proper orientation for hatching (Tullet and Deeming, 1987).

If eggs are not turned, hatchability is reduced (Landauer, 1961). Therefore, egg turning during incubation is essential for optimum hatchability (Tullet and Deeming, 1987). Eggs should be turned a minimum of three times per day (24 hours), with the optimum being 96 times per day. In most current automatic incubators, eggs are turned hourly (Lundy, 1969; Wilson, 1991). Turning is critical during the first two-thirds of the incubation period (with the most critical period in chickens being between three to seven days; Tullet and Deeming, 1987). For Japanese quail turning should stop at day 14 or 15 (Shanaway, 1994).

Between three and seven days of incubation, subembryonic fluid formation occurs. A lack of turning results in the presence of a layered effect in fluid secretions, a condition that persists throughout the incubation period, resulting in improper

mixing of nutrients. (Deeming *et al.*, 1987). The region of the yolk containing the germinal disc tends to float to the top of the egg, so that each movement of the egg brings the germinal disc into contact with fresh nutrients. Therefore, failure to turn the egg during the period before development of embryonic blood circulation can deprive the embryo of nutrients and oxygen (Shanaway, 1994).

In addition, turning is important for the development of the chorioallantoic membrane, which is essential for proper respiratory function, thus ensuring the correct physiological environment for normal embryonic development (Tullet and Deeming, 1987). A lack of turning also prevents normal growth of the albumen sac, resulting in a disruption of albumen utilization via the amnion (Wilson, 1991). Additionally, turning prevents embryonic adhesion to the inner shell membrane and to the yolk (Wilson, 1991).

Turning of eggs also influences hatching time; unturned eggs hatch later than eggs that have been turned throughout the incubation period (Tullet and Deeming, 1987).

Incubator Conditions

Several incubator conditions can also affect hatchability: incubator temperature, relative humidity (RH), airflow, and gaseous air composition. Additionally, there may be interrelationships between these factors, so that all must be continuously monitored.

Temperature

"The avian embryo depends on heat from an external source to maintain its temperature at a level conducive to rapid growth and physiological development" (Spiers and Baummer, 1990). Incubation temperature is considered by Wilson (1991) to be the most critical controllable environmental factor affecting hatchability. Landauer (1961) notes it to be of "prime importance for satisfactory hatching results." The effect of incubation temperature on hatchability depends upon how much the temperature deviates from the optimum, the duration of exposure to a particular temperature, and the age of the embryo (Lundy, 1969). There exists an optimum temperature at which the embryo develops most efficiently, thus maximizing hatchability (Wilson, 1991). Barott (1937) determined 100 F to be optimum, advising that it should not vary by more than ±0.5 degree F (0.28 C). The optimum incubation temperature for forced-draft incubators is between 99 and 100 F (Landauer, 1961). Research indicates that for optimum hatchability, the incubation temperature should be lowered to between 98 and 99 F during the last two days of incubation, with the amount of the reduction dependent upon the make of incubator (North and Bell, 1990). Reported optimum incubation temperatures have been established for "average" eggs, as the best temperature differs for individual eggs due to factors such as egg size, shell quality, genetics (including breed and strain), storage time, and relative humidity (North and Bell, 1990). The optimum incubation temperature varies with the species of bird, as well as the developmental stage during incubation. For instance, during the latter part of

incubation, ring-necked pheasant embryos are less sensitive to low incubation temperatures, while bobwhite quail embryos become less sensitive to high temperatures (Romanoff, 1934). For Japanese quail, the recommended incubation temperature for the first 14 days is 37.5 C (99.5 F). On day 15, the eggs should then be transferred to a hatcher set at 37 C (98.6 F; Sainsbury, 2000).

Byerly (1938) reported that mortality was about five times that of normal in eggs when incubated at low (97 F) or high (102.5 F) temperatures in forced draft incubators during days 5 to 17 of incubation, and 3.5 times normal at the low temperature and almost seven times normal at the high temperature during the last four days of incubation. During the first four days of incubation, hatchability was only slightly affected by either high or the low temperatures. He also reported an increase in malpositions, especially head-in-small-end, for both treatment groups. Temperatures that are too high or low may cause yolk absorption to be less efficient, as well as increasing the numbers of crippled chicks (Romanoff, 1934).

When eggs of precocial species undergo long-term exposure to ambient temperatures (below normal incubation temperature), hatching is delayed, and in *Coturnix*, embryonic growth rate is decreased by periodic cold exposure (Spiers and Baummer, 1990). According to Alsop (1919), incubation at low temperatures can result in abnormal development of the neural tube in very young embryos (24 hours of incubation), but by 72 hours of incubation, most embryos appeared normal, but many had fewer somites than normal. In Japanese quail, low

incubation temperatures result in large mushy chicks that typically have egg contents smeared on the down (Hoffman, undated).

Although somewhat resistant to lower incubation temperatures, developing chicken embryos are more sensitive to elevated temperatures above the optimum (Landauer, 1961). A lethal high incubation temperature (defined as that which killed approximately 50 percent of the exposed embryos) in the chicken ranges from 119 F pre-incubation to 108 F on the fourth and fifth days of incubation, and from 114 to 118 F for the remainder of the incubation period (Moreng and Shaffner, 1951). Barott (1937) reported that nearly all embryos died at an incubation temperature of 103.5 F. Tullet (1995) states that temperatures above 40.5 C (104.9 F) are lethal to chicken embryos, and Morgan and Tucker (1967) reported high embryonic mortality for three-hour exposures to a temperature of 41 C (105.8 F) at seven, eight, nine, or ten days of incubation. Increasing incubation temperature increases the incidence of various embryonic malformations—arrested gastrulation (Deuchar, 1952) and the presence of extra somites (Alsop, 1919). The time at which exposure to elevated temperature occurs influences which embryonic structures are affected. Brain and neural tube abnormalities have been observed when the temperature was elevated during the first three days of incubation, eye abnormalities during the first six days, and heart and kidney enlargement after a one-week exposure to 42.5 C (108.5 F; Wilson, 1991; Alsop, 1919). In *Coturnix*, incubation temperatures at or above 103 F (39.4 C) result in reduced hatchability (Padgett and Ivey, 1959). Turkey embryos incubated at 38.5

C (101.3 F) exhibited a high incidence of head-in-small-end malpositions, excess albumen, ruptured yolk sacs, edematous heads, eye cataracts, subcutaneous hemorrhage, and swollen down plumules (French, 1994).

Developmental processes of the embryo are accelerated with elevated incubation temperature, while decreased temperatures retard these processes. Growth rate profoundly influences incubation temperature, with arrested development occurring at 95 F (35 C, a very low temperature), favorable development at temperatures between 95 and 102 F (35–38.9 C), while temperatures above 102 F at first lead to accelerated growth, which is subsequently arrested (Romanoff and Faber, 1933; Barott, 1937). A temperature increase (within non-lethal ranges) before 13 days of incubation results in increased embryonic growth (Wilson, 1991). Time of hatch is accelerated at elevated temperatures, while it is delayed at lowered temperatures (Spiers and Baummer, 1990). *Coturnix* eggs incubated at 36.5 C (97.7 F.) hatched later (460 hours) than those incubated at 38.2 C (100.8 F; 390 hours; Mirosh and Becker, 1974). The metabolic rate of a chick embryo is partially the consequence of incubation temperature. Higher temperatures accelerate growth and *vice versa.* When the incubation temperature is optimal, the reversible metabolic reactions are in balance (North and Bell, 1990). Christensen *et al.* (2001) observed that increasing incubation temperature from 36.8 to 37.2 C (98.2 to 99.0 F) during the last three days of incubation led to changes in turkey embryos' growth rate. Observed physiological effects included elevated concentrations of insulin-like growth factors, plasma glucose, and glucagon at the

higher temperature. Embryonic thyroid function is also affected by incubation temperature, with depressed triiodothyronine (T_3) to thyroxine (T_4) ratios observed in embryos incubated at a lower incubation temperature (Christensen *et al.*, 2002).

Relative Humidity

Relative humidity (RH) in the incubator regulates the evaporation of water from the eggs while maintaining a proper physicochemical equilibrium among the egg constituents. Egg moisture loss is inversely related to incubation RH (Peebles *et al.*, 2001a). High humidity reduces evaporation of water through the eggshell, by occluding the pores, thus asphyxiating the embryo by preventing normal gas exchange, while low humidity permits excessive evaporation, which dehydrates the egg (Romanoff, 1934). One method of calculation of RH is by comparison of wet-bulb and dry-bulb temperatures (North and Bell, 1990). A wet-bulb thermometer is an ordinary thermometer in which a water-moistened wick has been placed around the bulb. Evaporation of the water from the wick cools the bulb, the amount of which is controlled by the humidity in the air. North and Bell (1990) contains a table listing relative humidities at different wet- and dry-bulb temperatures.

Barott (1937) observed that chick hatchability was optimal with 61% RH at 100 F. Hatchability decreased as the RH was varied in either direction from the optimum, but the RH may vary by as much as 10% from the optimum without excessively detrimental effects. The recommended RH for the hatcher is about 65% (North and Bell, 1990). Recommendations of optimal RH for *Coturnix* vary. Sainsbury

(2000) recommends 87% RH (wet bulb 30.6 C or 87 F) at a dry bulb temperature of 99 F and 90% (wet bulb 32.3 C or 90.1 F) at 37 C (98.6 F), while Shanaway (1994) recommends a RH of between 65 and 72% at 37.7 C (99.9 F), and Woodard *et al* (1973) recommend a wet-bulb temperature of 87 F (30.6 C) at an incubation temperature of 99.5 F (37.5 C) in the setter, and a wet-bulb temperature of 90 F (32.3 C) in the hatcher set at 99.5 F (37.5 C).

Air Flow and Composition

Adequate ventilation is essential in the incubator, as livability is jeopardized in the presence of high concentrations of carbon dioxide (CO_2; Decuypere *et al.*, 2001). In 1914, Lamson and Edmonds (as cited by Barott, 1937) reported that the best hatch was obtained when CO_2 concentrations in the incubator were at 0.5 percent, with hatchability decreasing as CO_2 concentrations were increased. In 1933, Romanoff and Romanoff (as cited by Barott, 1937) reported that increasing CO_2 concentration proportionally reduced oxygen (O_2) concentration. At concentrations of 0.4 percent CO_2 and 20.8 percent O_2, embryonic development was not significantly affected, whereas at 6.0 percent CO_2 and 19.6 percent O_2 no embryo survived beyond 2 weeks of incubation

The oxygen requirement of the embryo increases as the embryo advances in age and more carbon dioxide is liberated (North and Bell, 1990). Barott (1937) observed that it is essential that O_2 concentrations not be allowed to fall below the concentration in normal air, which according to North and Bell (1990) is about 21% at sea level. A five-percent oxygen deficiency reduced hatchability by nearly

one-third (from 81 to 55%). Barott also recommended that CO_2 concentrations should be kept below 0.5%, as concentrations higher than this were detrimental to embryonic development. CO_2 concentrations above 0.3% in the setter reduce hatchability, while a level of 5.0% is completely lethal, whereas the tolerance level in the hatcher is 0.75% (North and Bell, 1990). For Japanese quail, a drop in hatchability of four to five percent can be expected for every one-percent drop in the O_2 concentration below 18% (Shanaway, 1994).

Interrelationships Between Incubation Factors

Environmental conditions during incubation are not independent of each other, but each may have its own optimum for hatchability and chick quality (Decuypere *et al.,* 2001). Although many of these factors are of minor significance when taken separately, their effects can be cumulative, adding up to greatly reduced hatchability (North and Bell, 1990). Optimum temperature varies inversely with changes in relative humidity (Wilson, 1991). Barott (1937) observed that as incubation temperature was increased, it was necessary to decrease relative humidity. For instance, the optimum RH at 100 F. was 61%, but at 102, the optimum RH was 58%. Chicken embryos are unable to withstand high temperature and high humidity simultaneously (North and Bell, 1990).

Vick *et al.* (1993) reported that relative humidity (expressed as wet bulb temperature) should be varied as breeder hens age. As breeder hens age, physical characteristics of hatching eggs change, including a decrease in eggshell quality, which can be counteracted by slight increases in RH (Peebles *et al.*, 2001b). Vick

et al. (1993) also observed an interaction between breeder flock age and the wet bulb temperature during incubation, where lower hatchability was observed in some larger eggs from an older flock at a lower wet-bulb temperature, suggesting that a lower wet-bulb temperature may overcome barriers to water loss and gas diffusion in smaller eggs from young breeders. Additionally, there appears to be a storage temperature x storage time interaction, where higher temperatures (*i.e.* around 13 C or 55.4 F) are favorable for short-term stored eggs and lower temperatures (around 10 C or 50 F) are preferable when eggs are stored for longer periods (Proudfoot, 1969).

Genetics

Not all factors affecting hatchability are environmental—substantial genetic components contributing to variability in developmental parameters, as well as gene-environment interactions exist (Decuypere *et al.,* 2001). "Numerous hereditary factors are known to play a role in hatchability" (Landauer, 1961). Lethal and semi-lethal genes result in embryonic death and reduce hatchability (Funk and Irwin, 1955). Yoo and Wientjes (1991) observed a difference in the decline in hatchability (primarily due to an increase in early embryonic mortality) as storage time (above three to four days) increased between related strains of Australorp chickens. Their findings suggested a genetic difference in ability to tolerate longer storage times. The authors further suggested that embryos that are susceptible to increased storage time might also be more vulnerable to unfavorable storage conditions in general.

The optimal range of incubation conditions such as temperature may be altered by selection for egg production as compared to meat production (Decuypere *et al.*, 2001). Survivability of turkey embryos incubated at higher than normal temperatures (37.3 C or 99.14 F) from days 25 to 28 differs, depending on whether the genetic lines used are selected for egg production or growth, with egg-selected lines demonstrating altered organogenesis, while growth-selected lines demonstrate deceleration of growth (Christensen *et al.*, 1999; Christensen *et al.*, 2001). Selection of turkeys for faster growth rate may also result in reduced hatchability, due to altered energy metabolism. Rapidly growing embryos were heavier at hatch, with elevated plasma glucose levels and organ glycogen concentrations, which appear to be related to decreased livability (Christensen *et al.*, 2000).

Hatchability declines during the development of inbred lines of chickens (Crittenden and Bohren, 1962). Likewise, *Coturnix* quail appear to be very sensitive to inbreeding, in that adverse effects of inbreeding on hatchability, viability, and egg production have been found to be almost twice as severe in quail as in chickens or turkeys (Baumgartner, 1993). The pronounced inbreeding effects observed in Japanese quail populations would suggest that *Coturnix* quail carry many harmful recessive genes which are uncovered as homozygosity increases due to inbreeding (Woodard *et al.*, 1973).

In turkeys, hatchability is genetically related to variation in several egg-quality traits (Nestor *et al.*, 1972), including egg weight, albumen height, shell weight, percent shell weight loss during seven days of incubation, and percent incubation weight loss, wet weight of yolk, percent yolk, and percent dry matter in the yolk. Twenty-five percent of the variation in hatchability of fertile eggs can be explained by variation in nine egg quality traits, and shell weight is positively correlated with hatchability late in the laying season.

Lethal genes have a significant effect on hatchability, since genetic defects increasing embryonic mortality concomitantly decrease hatchability (Card and Nesheim, 1972). Landauer (1961 and 1973), Savage *et al.* (1988), Savage (1990), and Somes (1990) describe a range of lethal genes affecting poultry. Cheng and Kimura (1990), Tsudzuki *et al*, (1998), and Kubota *et al*, (1995) likewise describe lethal genes observed in *Coturnix*. Work is currently in progress at Oregon State University to identify other previously unreported lethal genes in Japanese quail (Savage *et al*, 2003).

Development Time

Avian development time may be affected by several factors, including species, environmental and genetic factors, and a phenomenon known as synchronization of hatching. On average, different poultry species have different development times (incubation periods). At 100 F the incubation period for Japanese quail is 16 days ± 8 hours (Padgett and Ivey, 1959) or 16.4 days at 99.5 F in the setter and 98.5 F in the hatcher (Abbott and Craig, 1960). At normal incubation

temperatures, the average incubation period is 21 days for chickens, 28 days for turkeys, 23-24 days for pheasants, 23 days for bobwhite quail, (Mercia, 1980), and 29 to 32 days for geese (Sainsbury, 2000).

Several environmental factors that may influence embryonic development time are breeder age, storage time, and conditions within the incubator. Incubation time decreases as broiler breeder hens advance in age (Peebles *et al.*, 2001a). Crittenden and Bohren (1962) conducted an experiment suggesting that eggs of younger pullets may require a longer time to hatch, finding that "in young pullets of varying ages, the older birds produce eggs that hatch better and earlier," and that the longer a pullet has been in production, the shorter the incubation period.

Egg storage time may influence development time. *Coturnix* eggs stored for 13-21 days hatched an average of 13.3 hours later than those stored for 1-4 days (Mirosh and Becker, 1974). Increased egg storage results in longer incubation times; storage for one additional day adds one hour to the incubation period (Mirosh and Becker, 1974; Decuypere *et al.,* 2001). In chickens, Mather and Laughlin (1976) observed that storage of eggs for 14 days increased the mean total incubation period by 13.4 hours.

One of the most important factors that influence the length of avian embryonic development is incubation temperature. According to Barott (1937), chick embryos incubated at 100 F hatched at about 20.25 days, with a range from 19.2 days at 103.5 F to 23.5 days at 96 F. Romanoff (1936) and Romanoff and Faber

(1933) observed that development time is retarded by lower incubation temperatures and advanced by higher temperatures. Temperatures above 39.5 C delayed hatching time. In an experiment utilizing Rhode Island Red chickens, embryonic development in eggs incubated at 36 C lagged about 10 hours behind those incubated at 38 C (Al-Thani and Simkiss, 1992).

Periodic interruptions in incubation may also affect development time; Callebaut (1990) reported that consecutive daily eight-hour interruptions in incubation resulted in lengthening of the incubation period by one-third without apparent harm, and a possible improvement of hatchability in Japanese quail.

Relative humidity in the incubator may alter development time. In Romanoff's (1934) experiments with pheasant and bobwhite quail, eggs of both species tended to hatch earlier at a low humidity and later when the humidity was high. Romanoff (1938), in a subsequent study, also noted that rapid air movement within the incubator hastened the time of hatch, whereas hatching time was retarded by slow air movement. This difference he attributed to the rate of evaporation at the different air velocities.

Lighting within the incubator may also influence development time. Several studies have indicated that chick embryo development rate can be accelerated by illumination with both incandescent and fluorescent lighting. Illuminated embryos were larger at 15 days of incubation and hatched an average of five hours earlier than the controls (Lowe and Garwood, 1977).

Although environmental factors play a major role in development time, genetic factors within a breed or species have substantial effects on the length of time required for incubation. Development time and hatchability are known to differ among genetic strains when environmental factors are controlled, with unspecified genetic factors being credited with these results. Since hatchability depends on many factors, no specific "hatchability gene" has been discovered (Card and Nesheim, 1972). Brake *et al.* (1997) noted that albumen quality, which can affect hatchability and incubation time, differed in seven different strains of broiler hens, In addition, embryonic development at oviposition has been observed to be different in different genetic lines, which is probably the effect of the genetically determined rate of early cell division and development (Decuypere *et al.,* 2001). Additionally, female chicken embryos were observed to hatch an average of three hours earlier than male embryos in unstored eggs incubated at 99.5 F. (Mather and Laughlin, 1976).

Synchronization of hatching occurs in many precocial bird species, including domestic fowl, ducks, bobwhite quail (*Colianus virginianus*), and Japanese quail. In some species (*i.e.*, chickens and ducks), signals from more advanced embryos accelerate the development of those that are less advanced, so that they all hatch at about the same time. In other species (*e.g.*, both quail species listed above), the less advanced embryo can also retard the development of the more advanced embryo, so that the older embryos hatch later and the younger ones hatch earlier than they normally would (Grieve *et al.*, 1973). In Japanese quail, hatching can be

advanced by as much as 32 hours by synchronization (Grieve *et al.,* 1973). This embryonic "communication" utilizes both audible clicking noises and low frequencies just below the auditory threshold (Freeman and Vince, 1974). These clicking noises are the result of embryonic breathing during the final stage of incubation. For synchronization to occur, the eggs must be in close contact with each other (Vince, *et al.*, 1984). If quail eggs are separated by a distance of even a few inches, hatching occurs over an extended period of time, whereas if the eggs are in contact with each other, they will hatch within an hour or two of each other (Freeman and Vince, 1974). Chicks that have hatched early due to synchronization are equally mature as chicks hatched in the normal time, but livability may be reduced (Grieve *et al.,* 1973).

Post-hatch Growth and Livability

Post-hatch growth and livability, defined as the percentage of broiler chicks alive at the end of the time they are sent to market (Sainsbury, 2000), can be affected by many factors, such as maternal nutrition (Funk and Irwin, 1955) and incubation temperature (Kühn *et al*, 1982; Wilson, 1991). Although little or no difference in post-hatch mortality to three weeks of age was observed in chicks that hatched from eggs incubated at different temperatures, game birds and turkeys are much more susceptible to post-hatch mortality than White Leghorn chicks when incubated under improper conditions (Romanoff, 1936). Chick quality may be affected by abnormal incubation temperatures. Severe heat stress during incubation has led to weaker chicks, with a higher incidence of culls as the severity

of heat stress increased. These chicks also exhibited a high incidence of clubbed wiry down and walked with an unsteady gait (Wilson, 1991). Eggs incubated at high temperatures were observed to produce a greater incidence of crippled chicks. Chicks incubated at 102 F (38.9 C) also expressed greater incidences of crooked or curled toes, sprawling legs, and cooked necks (Romanoff, 1936). These chicks were smaller, less lively, and not as fluffy as those incubated at 100 F (37.8 C). (Barott, 1937). Other consequences of heat stress for various amounts of time and at different stages of incubation included reduced hatchability, as well as an increased incidence of cull chicks—weaker and less alert than normal chicks with curled toes, weak legs, general lack of balance and an unsteady gait, and wiry and/or matted down (Thompson *et al.*, 1976; Ande and Wilson, 1981). In Japanese quail, high incubation temperature symptoms include lightweight chicks with desiccated legs and feet and/or curled toes that cannot be extended (Hoffman, undated).

Incubation temperatures can also influence postnatal growth (Kühn *et al.*, 1982; Wilson, 1991) and development in chickens and turkeys, including their subsequent age at onset of egg production, which was delayed by three days when the embryo was incubated at 33.8 C (99.5 F), compared to 37.8 C (92.8 F). Mean egg weight was also significantly reduced in the 33.8 C group (Decuypere *et al.*, 1985). Incubation at low temperatures (33.8 C. or 92.8 F.) has shown an increased post-hatch weight loss and reduced body weight, due to reduced circulating plasma triiodothyronine (T_3) levels, as well as leading to increased mortality during the

first six weeks post-hatch (Kühn *et al.,* 1982). Incubation at 32.5 C. (90.5 F.) can result in enlargement of the heart, even though body weight is less than that of the controls incubated at 37.5 C. (99.5 F.; Leighton *et al.,* 1964). Chicks incubated at 35.8 C (96.44 F) for the first 10 days of incubation and at 37.8 C (100 F) for the remainder of the incubation period exhibited compensatory growth, both before and following hatching. This compensatory growth persisted up to the reproduction period, which suggests a link between environmental conditions, such as incubation temperature, growth processes, and metabolic level (Geers *et al.*, 1983).

Parthenogenesis

Parthenogenesis is defined as "The development of eggs without fertilization" (John, 1990), or "Reproduction without the genetic participation of sperm" (Markert and Seidel, 1981). This phenomenon occurs in nature in most animal phyla, including vertebrates. Parthenogenetic development has been observed in chickens (Olsen and Poole, 1962) and turkeys (Olsen, 1965; Savage and Harper, 1986), and is usually manifested in several different stages, classified as: unorganized membranes, ruptured vitelline membrane (yolky), blood formation, and embryo formation (Yao and Olsen, 1955; Harada and Buss, 1981; Savage and Zakrzewska, Undated). Parthenogenesis occurring at any of these stages can be incorrectly identified as early embryonic failure (Savage and Zakrzewska, Undated). Once in a while, a living parthenogenetic poult will hatch and attain sexual maturity. Olsen (1965) observed that about seven of every 100 parthenotes

survived to the hatching stage, and over 25 matured and produced semen containing viable spermatozoa. In turkeys, parthenogenesis has been observed to be negatively correlated with fertility and semen volume (Savage and Harper, 1986).

Genetic Selection Studies for Improvement of Hatchability

Genetic selection can be applied to incubation traits, such as hatchability, although the rate of change may be slow (Hutt, 1969). Hatchability is determined by a "complicated genetic constitution" (Warren, 1953).

Hatchability is generally considered to be a trait of the parents (Besbes and Ducrocq, 2003), as either the sire or the dam (or both) may be responsible for embryonic failures (Hays and Klein, 1952; Warren, 1953); both sire and dam contribute to the inheritance of high hatchability (Hays and Klein, 1952).

Since high hatchability is equivalent to the absence of lethal embryonic factors, selection for improved hatchability is actually selection against lethal genes (Hays and Klein, 1952). In order to prevent reduced hatchability, lethal and subvital (semi-lethal) genes need to be recognized as such and eliminated from the breeding population. The source of lethal genes should be identified among the pedigreed parents or grandparents, and eliminated by appropriate testing (Cole, 1969). Genetic aspects of hatchability are not necessarily limited to the effects of lethal genes. There is evidence to indicate that the genotype of the dam is implicated in the hatchability of her eggs—especially in relation to traits such as

egg or shell quality, which would include genetic nutritional deficiencies in the egg (Hutt, 1969). Genes for certain plumage colorations are known to adversely affect hatchability (Hutt, 1969).

It is well established that inbreeding may reduce hatchability (Düzgünes, 1950; Hays and Klein, 1952; Hutt, 1969), presumably by increasing homozygosity for recessive lethal gene expression. This phenomenon, inbreeding depression, is defined as "The reverse of hybrid vigor—a decrease in the performance of inbreds, most noticeably in traits like fertility and survivability" (Bourdon, 2000). A means of counteracting this is by utilization of hybrid vigor (or heterosis) defined as "An increase in the performance of hybrids over that of purebreds, most noticeably in traits like fertility and survivability (Bourdon, 2000), which is gained by crossing of breeds, strains, or inbred lines, leading to heterozygosity (Hays and Klein, 1952; Hutt, 1969). For hatchability, heterosis is a trait of the individual, that includes a substantial contribution from the dam (Fairfull and Gavora, 1993). According to Woodard, *et al.*, (1973), Japanese quail exhibit a pronounced susceptibility to inbreeding depression, expressed as adverse effects on hatchability, viability and egg production. For this reason, breeding systems of *Coturnix* should not include brother x sister matings.

Limited selection studies to improve hatchability in the chicken and turkey have been successfully conducted. From 1933 to 1940, selective breeding in Rhode Island Red hens in Massachusetts improved hatchability from 61 to 75% (Hays and Klein, 1952). Additionally, selection for reduced embryonic mortality in the

turkey at three, four, and six days of incubation can be accomplished, as reported by Christensen in 1978 (as discussed in Smith *et al.*, 1991), however, a reduction in mortality between days 24 and 28 of development was not observed

The Use of Japanese Quail (*Coturnix japonica*) as a Pilot Species

Often, poultry research is restricted by budgetary limits, as well as limits on time and space. For this reason, it may be advantageous to utilize a pilot species that is easy to handle, hardy, and possesses a short generation turnover, and is less costly to manage. One such species is the Japanese quail (*Coturnix japonica*). The female lays its first eggs at about five to six weeks of age, thus allowing at least four generations in one year. Sex determination is possible at as early as three weeks of age. Quail maintenance costs are lower in terms of space and feed (eight to ten quail can occupy the same amount of space as one chicken and 30 quail occupy the space required for one turkey (Abbott, 1967; Woodard *et al.*, 1973). Japanese quail possess a physiological resemblance to chickens and turkeys, thus allowing for analogous results (Wilson *et al.*, 1961; Woodard *et al.*, 1973). Japanese quail are also used in biomedical research, biological research, and space research (Minvielle, 2004).

Materials and Methods

Incubation of Eggs

In order to establish a base population, fertile Japanese quail eggs were obtained in June of 2001 from a randomly mating flock maintained at Oregon State University. A total of 400 eggs were randomly assigned to one of two groups, designated as Control (Line C) and Selected (Line S). Both groups were placed in "identical" Jamesway 252 single stage incubators, which had been adjusted to 37.8 C (100 F) for Line C and 38.9 C (102 F) for the high temperature treatment group (Line S), respectively. Relative humidity was maintained at 30 C (86 F) wet-bulb. On the seventh day of incubation, the eggs were removed from the incubators and candled to detect infertile eggs and early dead embryos. The contents of all eggs not exhibiting development were examined macroscopically to determine fertility and/or age at and cause of embryonic death (Padgett and Ivey, 1960). The percent of fertile eggs was determined at this time. On day 14 of incubation, the eggs of both groups were transferred to pedigree hatching baskets and placed in a third Jamesway 252 hatcher (98.5 F dry bulb and 88-90 F wet bulb temperatures; Shanaway, 1994). The eggs remained in this unit for two (Line S) or three days (Line C), at which time the chicks were removed from the incubator (Line S chicks hatched about one day earlier than Line C chicks). The contents of all eggs that did not hatch were macroscopically examined and recorded as either "pipped" or "dead-in-shell"(D3). The percentages of early dead embryos (D1, days one to seven), mid-deads (D2, days eight to 14), and late deads (D3, days 15 onward),

and embryos that had broken the eggshell but had not hatched (pips) were calculated, as well as percent hatch of fertile eggs (HOF) set. Ages of unhatched embryos were determined as described by Padgett and Ivey (1960). Eggs were incubated weekly for a total of four consecutive sets to ensure adequate numbers of birds for breeding (if a single set were inadequate) and to provide additional hatchability and embryonic mortality data. Commencing with the second generation, identical procedures were followed, except that Line S eggs were set in the incubator one day later than Line C eggs in to permit all chicks to all hatch on the same day.

Brooding and Rearing of Chicks

Subsequent to the hatch of chicks, those to be used as potential breeders were wing-banded for pedigree identification purposes. The chicks were then randomly placed in stackable 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®). An eight-hour light:16-hour dark lighting schedule was maintained in the brooder room. 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)

(Woodard *et al.,* 1973)

At four weeks of age, all surviving birds were identified by wing-band and sex, and body weight, and any defects (if present) were recorded. Birds were also beak-trimmed at this time in order to minimize cannibalism (Woodard *et al.*, 1973; Shanaway, 1994), separated by line and sex, and placed in colony-style holding cages at ambient room temperature until breeder candidate selection had been completed. At this time the birds' diet was changed to Purina® Game Bird Layena® (Complete Ration), which was fed for the balance of that generation, and the lighting schedule was increased to sixteen hours of light per day.

Selection of Breeding Pairs

About five weeks of age, those females selected as breeders were transferred to individually numbered breeder cages. In generations 2, 3, 4, 6, 7, 8, and 9, female breeders were isolated in their respective breeder cages until sufficient "virgin" eggs had been laid to conduct a survey of parthenogenesis in *Coturnix*. The males were subsequently introduced into the breeder cages. Alternate breeders were maintained in case a replacement breeder was required, *e.g.*, due to mortality. Egg records for each cage were maintained to calculate the onset of sexual maturity and

hen-day egg production. Once the hens were at maximum production, eggs required for reproduction of the lines and incubation data were collected and marked daily with the cage number on the egg and subsequently stored in a common egg holding facility maintained at 12.8-15.6 C (55-60 F) for a maximum of 12 days for the first set of each generation, and usually for no more than seven days for subsequent settings. It is possible, however, that egg storage temperatures may have been higher in summer months.

Each line was composed of 25 breeding pairs of birds placed in cages numbered from one to 50. The birds from each line were interspersed in the breeder cages such that all the odd-numbered cages (*i.e.,* 1, 3, 5, ...) were reserved for the Line S breeders and the even-numbered (*i.e.,* 2, 4, 6, ...) for Line C. The selection criterion for Line S breeder candidates was based upon the mean hatchability performance data for the four consecutive egg settings in each generation.

Control (Line C)

Selection of breeders for the randomly mated line was based upon the effort to select one male and one female from each paired mating without regard to the hatchability performance. The assignment of the male and female breeders was at random, except that brother-sister matings were avoided to reduce the likelihood of inbreeding. Twenty-five paired matings were established in each generation of the study.

Selected (Line S)

Selection of breeders for the Selected population was based upon the hatchability of the birds' breeder parents as compared to the overall mean hatchability of the line in that generation. Breeder candidates were selected from offspring from those pair matings in which their mean percent hatchability exceeded the overall mean for Line S. An effort was made to maintain a selection differential of at least +10 percentage units. Due to this applied selection pressure, it was common practice to save two or three male or female birds from the superior families. There was no effort to apply selection pressure for improving fertility or reducing embryonic mortality by category. The assignment of the breeders to the cages was such that full-sib matings were avoided. This procedure was repeated for 10 consecutive generations. Twenty-five paired matings were established in each generation of the study.

Performance Parameters Studied

During the course of this study a series of performance traits and incubation characteristics were measured, including:

Body weights: at four to five weeks of age, for generations 1, 2, 3, 4, 5, 6, and 10.

Onset of egg production (sexual maturity of the hens—the ages at which the hens laid their first eggs) and egg production were recorded for both lines in each generation.

Daily egg production by hen was also recorded in order that hen-day egg production could be calculated for the two lines. Hen-day production, a measure of a hen's productivity, is calculated as 100 times the number of eggs laid by an individual hen in a specific number of days (*i.e.*, if egg records were kept for three months, the number of days would begin with the hen's onset of lay and end at the end of the record-keeping period).

Egg weights by hen and line were also determined prior to incubation for all four settings in generations 1-10.

Early chick mortality (0-10 days of age): the numbers of chicks that died in the first 10 days following their emergence from the egg and subsequent placement in the brooder cages were recorded. This measurement was only recorded in the first hatch of each generation.

Incubation traits: percent true fertility of eggs set, early, mid, and late dead embryonic mortality, pipped eggs, and chicks that hatched (hatchability) were calculated for each of the four settings per generation and subsequently averaged over the four egg sets.

Parthenogenetic development

During generations 2, 3, 4, 6, 7, 8, and 9, eggs were collected and marked by cage prior to the introduction of the males. The eggs from the two respective lines were subsequently placed in their respective incubation environments (100 F or 102 F) for seven days. The eggs' contents were macroscopically examined for signs of parthenogenetic development. (Olsen, 1965).

Statistical Analyses

All data were entered into a computer database (Microsoft®Excel). Statistical tests were performed using the S-Plus® 4.5 statistical software package (Hinrichs *et al.*, 1999). Two-sided *t*-tests were conducted for each generation in order to compare body weights, egg weights, and age at onset of sexual maturity in the females (onset of lay) to determine differences between lines. The Bonferroni correction was applied to adjust rejection levels for family-wise variation (Ramsey and Schafer, 2002). This procedure generates confidence levels that are more conservative than the standard α=0.05 to which *p*-values are compared to determine if test results warrant rejection of the null hypothesis. Tests of differences of proportions were performed on all data recorded as percentages, *i.e.*, incubation data and hen-day production. Variances were calculated according to Ramsey and Schafer (2002). In order to compare egg weights, age at onset of lay, post-hatch mortality, fertility, incidences of D1, D2, and D3 embryos, pipped eggs, and hatchability of fertile eggs, Multiple Linear Regression (MLR) analyses were conducted with Line as an indicator variable with Line C as the reference group, since it is the control (Line S=1 and Line C=0). This model gives the following formulas for regression lines:

Line S: $y = (\beta_0 + \beta_2) + (\beta_1 + \beta_3)$*Generation, and

Line C: $y = \beta_0 + \beta_1$*Generation,

where β_0 is the intercept coefficient, β_1 is the coefficient for Generation, β_2 is the coefficient for Line, and β_3 is the interaction coefficient. The standard errors for

each coefficient are reported in parentheses beneath the regression line formulas. Results are included for those data sets for which a linear model was deemed appropriate as determined by multiple R^2 values, which, as the percentage of the total response variation explained by the explanatory variable(s), provide an image of the tightness of the fit of the regression model (Ramsey and Schafer, 2002). The residual standard error (SE), which is the estimated standard deviation around the regression, is also reported for each MLR.

Since data were collected over successive generations, one might question the assumption of independence. Including generation as an explanatory variable, however, accounts for this. Assumptions of normality were evaluated by examination of normal QQ plots and assumptions of equal variance by examination of residuals plots. Outliers identified as influential by Cook's Distance plots were examined in further detail, and in cases where results were of interest, graphical data plots are presented comparing the regression lines obtained utilizing the full data sets with those from which influential data points have been removed (Ramsey and Schafer, 2002).

Results and Discussion

In this chapter, observations of breeder performance traits of the Control (Line C) and Selected (Line S) lines are presented, followed by the hatchability traits studied.

Body Weights

Body weights of males and females were collected and recorded separately in generations 1, 2, 3, 4, 5, 6, and 10 at ages ranging from 4 to 5.3 weeks. These results are summarized in Figures 4.1 and 4.2, respectively. Statistical analyses (*t*-tests) were conducted separately for males and females in each generation, as body weight is a sexually dimorphic trait in Japanese quail, with the females being heavier than the males at as early as two weeks of age (Shanaway, 1994). Calculation of the Bonferroni correction for family-wise variation yielded $\alpha = 0.007$ (α is the probability of rejecting the null hypothesis when it is in fact true, and is the value used for determining whether a *p*-value justifies rejection of the null hypothesis in favor of the alternative hypothesis). Standard errors and *p*-values are summarized in Appendix Tables A1.1 and A1.2. All *p*-values are two-sided unless otherwise stated. In the males, body weights were significantly higher ($p<0.001$) in Line S in all generations except the first (Figure 4.1).

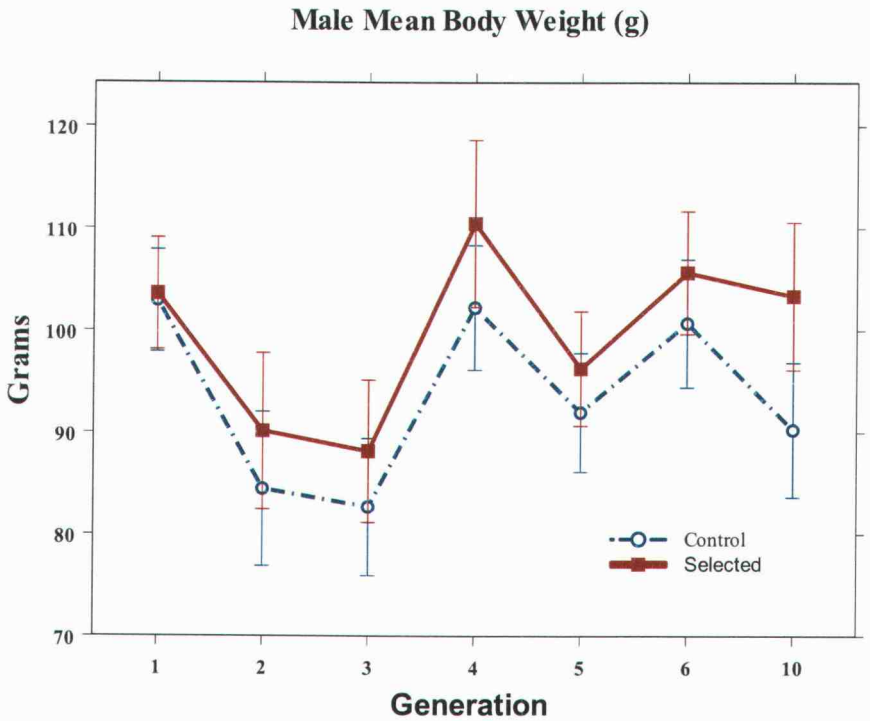

Figure 4.1 Comparison of mean body weights in grams of males of Lines C and S over 10 generations with standard error bars (the number of birds weighed ranged from 26 to 120). Body weights were not recorded in generations 7, 8, and 9.

Similar results were observed in the females, except that the Line S females were significantly heavier ($p< 0.001$) in all generations (Figure 4.2). Lower body weights were observed in those generations measured at younger ages (see Appendix Tables A1.1 and A1.2 for exact ages, standard errors, and *p*-values). In chickens, incubation temperature affects the subsequent thyroid status, growth rate, and reproduction of the hatchling (Wilson, 1991; Kühn *et al.*, 1982). Since chick weights at hatch were not determined, it remains unknown whether Line S chicks were larger at hatch than Line C chicks, maintaining this difference until

the time they were weighed at four or five weeks of age, or if Line S chicks grew at an accelerated rate compared to those in Line C. Since elevated incubation

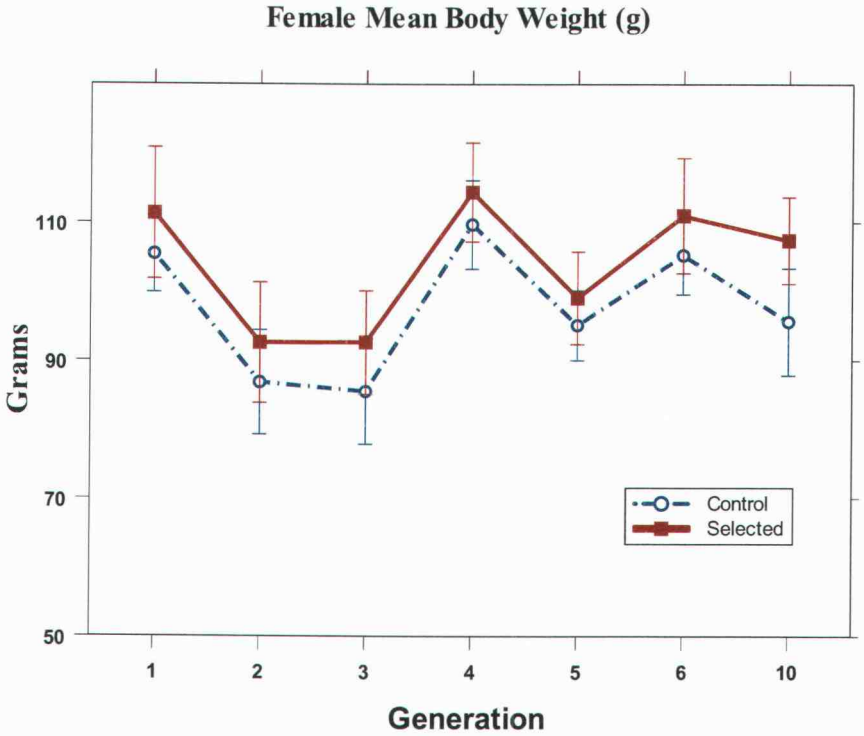

Figure 4.2 Comparison of mean body weights in grams of females of Lines C and S over 10 generations with standard error bars (the number of birds weighed ranged from 35 to 89). Body weights were not recorded in generations 7, 8, and 9.

temperature increases the pre-hatch embryonic metabolic and development rates (Wilson, 1991; North and Bell, 1990), it is possible that this acceleration is maintained post-hatch. Although it would have been of interest, determination of thyroid hormone levels and metabolic rates were beyond the scope of this experiment. It also would have been interesting to determine if the difference in body weights between lines persisted when the birds attained their full adult body weights at about 50 weeks of age (Shanaway, 1994). Determination of this would

be impractical, since any disturbance of breeder females can result in cessation of lay (Woodard *et al.*, 1973), and due to budgetary restraints, birds were only kept until approximately 14-16 weeks of age.

It is possible that selection for high hatchability at an elevated incubation temperature is in some way an indirect selection for increased body weight through a correlated response to selection (Bourdon, 2000), or there could be a genotype x environment interaction, in which the difference in performance between two genotypes (Line S and Line C) changes from environment to environment (high and low incubation temperatures (Bourdon, 2000).

Onset of Egg Production

The onset of female sexual maturity, manifested as the onset of egg laying for the ten generations of selection, was examined. Results are summarized in Figure 4.3 and Appendix Table A1.3. Calculation of the Bonferroni correction for family-wise variation yielded $\alpha = 0.005$. Time to onset of lay (in days) was significantly different in generation 3, in which Line S hens matured an average of 5.6 days earlier than Line C hens ($p<0.001$). In generation 5, Line S hens matured an average of 7.0 days later than the controls ($p<0.01$), and 4.4 days later in generation 7 ($p<0.01$). In generation 8 Line S hens were delayed by an average of 3.4 days as compared to Line C ($p<0.01$). In generation 5 onset of lay was delayed in both lines as compared to other generations. This particular generation hatched in July of 2002, and it is possible that hot weather may have exerted an environmental effect, as high ambient temperatures can affect egg production by

reducing the hen's appetite (Shanaway, 1994). Undetermined environmental effects appear to have influenced onset of lay across the generations, since the direction of the change between generations is generally the same. In generations 9 and 10, however, there was no difference in age at first egg between the lines, which could be indicative that genic segregation had begun to affect this trait in Line S. Sexual development is a complex process that is influenced by many factors, including age and body weight. It has been suggested that there may

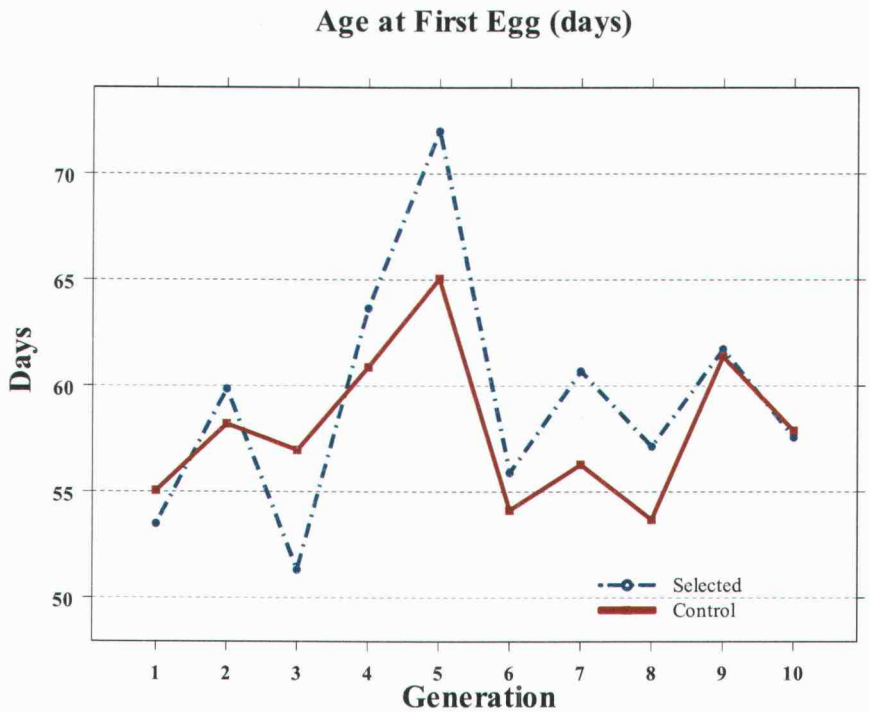

Figure 4.3 Comparison of age in days at onset of female sexual maturity between Lines C and S for the 10 generations of selection.

be multiple threshold traits involved in the onset of lay in Japanese quail, such as chronological age, a minimum body weight and composition, as well as growth rate (Zelenka *et al.*, 1984; Oruwari and Brody, 1988; Reddish *et al.*, 2003). It is

possible that Line S hens matured earlier because they reached a threshold body weight earlier than their Line C counterparts. Perhaps four-week body weight and/or growth rate are correlated with early sexual development. Since body weights were not recorded for each generation, correlations between these traits were not calculated. MLR analysis results are not included, as the data did not produce an adequate linear model (Multiple R^2=0.087).

Hen-Day Egg Production

Hen-day egg production data for the ten generations of selection are summarized in Figure 4.4 and Appendix Table A1.4. Calculation of the Bonferroni correction for family-wise variation yielded $\alpha = 0.005$. In generation 3 average hen-day egg production was somewhat lower in Line C than in Line S (p=0.051). However, in generations 5, 6, 7, 8, 9, and 10 hen-day production was significantly higher in Line C (p<0.001). Average egg production in Line S had declined by 8.9% since the beginning of the study (Figure 4.4). The results suggest that differences in hen-day egg production became more pronounced after generation 4, which may suggest that gene segregation may have been occurring within Line S. The egg production declined in both lines following generation 7, which suggests that there may have been some environmental factors affecting this trait. Line S, however, appeared to have been more responsive to these conditions. It is possible that time of year could have been one such environmental factor, as the birds hatched in February, May, August and December of 2003 for generations 7, 8, 9, and 10

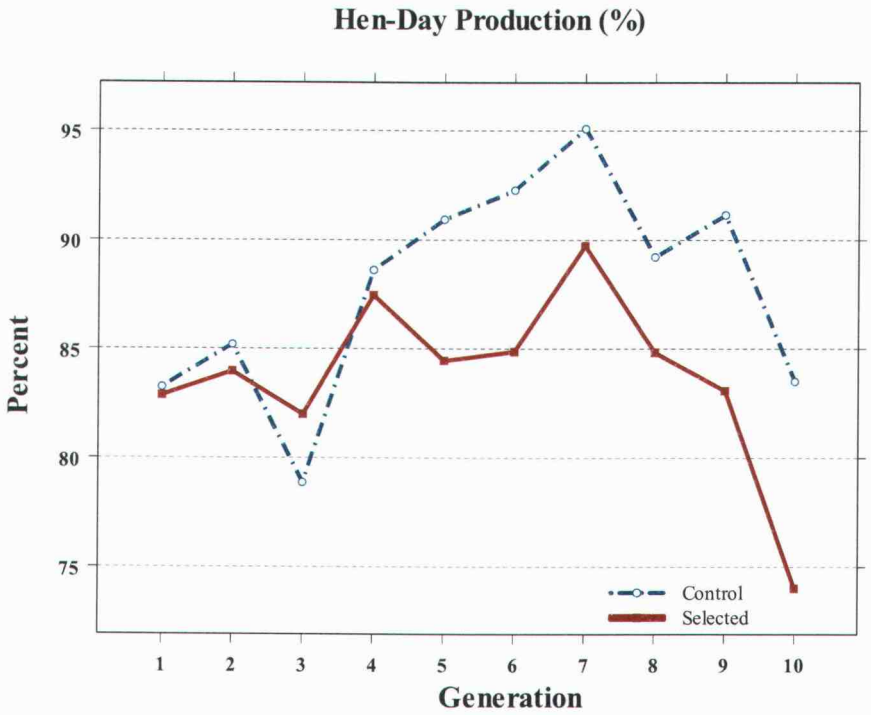

Figure 4.4 Comparison of percent hen-day egg production between Lines C and S over 10 generations of selection.

respectively, and temperature extremes are known to affect egg production (Card and Nesheim, 1972). The weather was exceptionally cold in late December, 2003 and early January, 2004. Although the facilities have a limited environmental control, extreme ambient temperatures cannot be completely counteracted.

MLR analysis was performed in order to compare mean percent hen-day production between Lines C and S over generations 1-10 (Figure 4.5). Hen-day egg production has decreased slightly over the generations in Line S, while increasing in Line C. This decline in egg production in Line S may be related to the observed increase in body weight, or it may be due to some mechanism of

genetic homeostasis, in which fitness traits (of which egg production is one) decline as genetic selection is practiced.

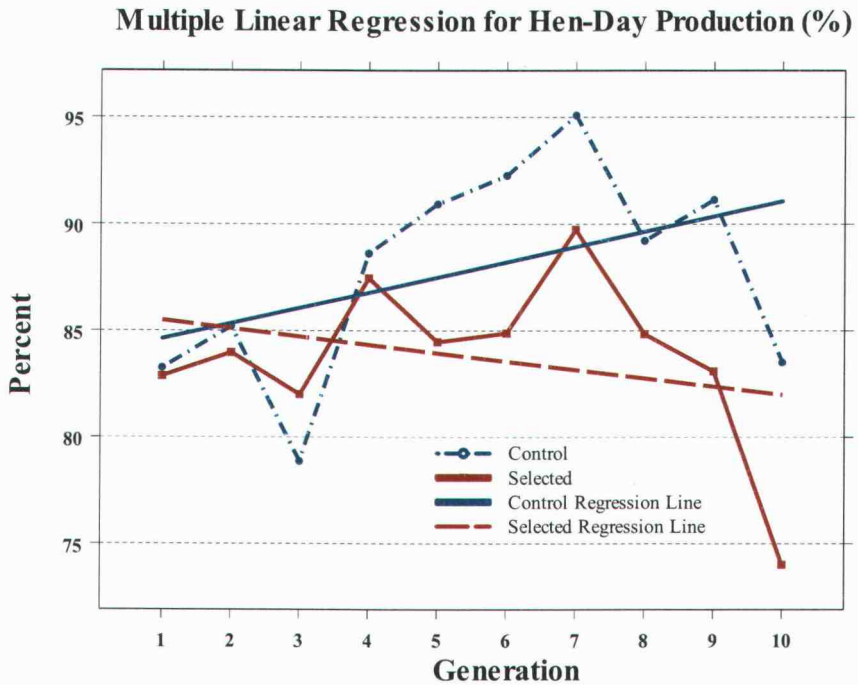

Figure 4.5 Multiple linear regression lines for percent hen-day production. The residual standard error is 4.472. Multiple R^2=0.30. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 83.91 + 0.71x$
(3.06) (0.49)
Line S: $y = 85.88 - 0.39x$
(3.06, 4.32) (0.49, 0.70)

Egg Weights

Egg weight data recorded for all 10 generations are summarized in Figure 4.6 and Appendix Table A1.5. Calculation of the Bonferroni correction for family-wise variation yielded $\alpha = 0.005$. In generation 1 there was no difference in egg weights between the lines as all eggs were obtained from a common pool. It should be

noted that the mean egg weight was considerably higher in the first generation, which may be attributed to breeder age, which is known to increase egg weight. At the onset of this study, the breeder population from which the founding eggs were obtained was over one year old versus the typical breeder age of about 8 to 14 weeks in generations 2 to 10.

With selection for incubation performance, fluctuations in mean egg weight were observed in Line S. These fluctuations could be attributed to unknown environmental factors, such as time of year, since the direction of change is the same between lines. Egg weights were significantly higher by an average of 0.36 g

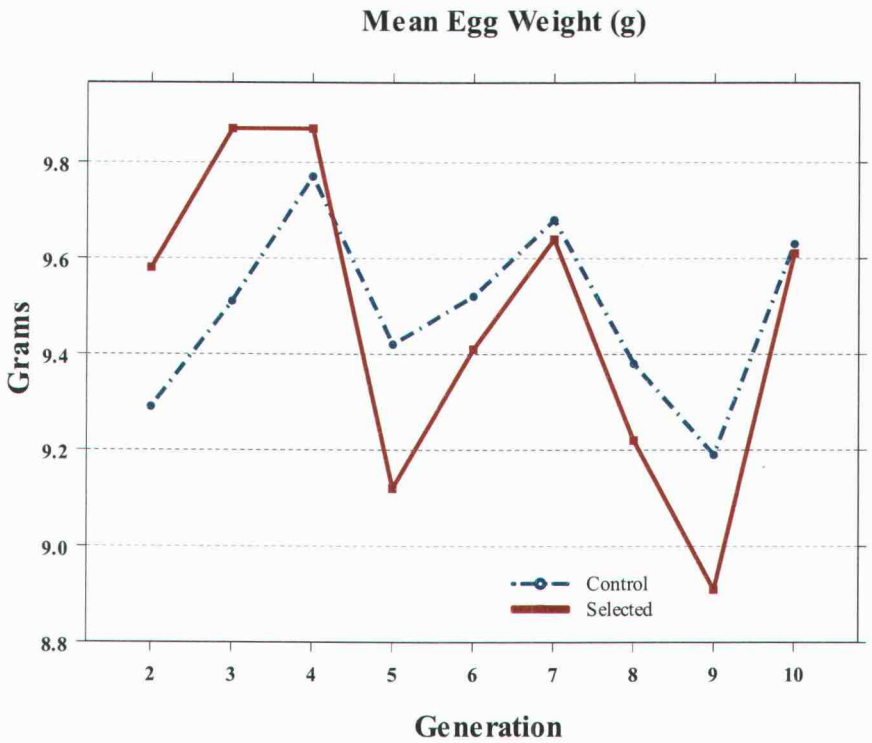

Figure 4.6 Comparison of mean egg weights in grams between Lines C and S over 10 generations of selection.

in Line S in generation 3 ($p<0.001$). Although egg weights were numerically higher in Line C from generations 4 to 9, none of the differences were significant.

MLR analysis for egg weights are contained in Figure 4.7. Generation 1 was omitted from the MLR analysis due to a potential breeder age effect. Egg weights fluctuated over the generations in both lines, with Line S declining overall, while Line C remained constant. The negative slope observed for Line S may reflect the

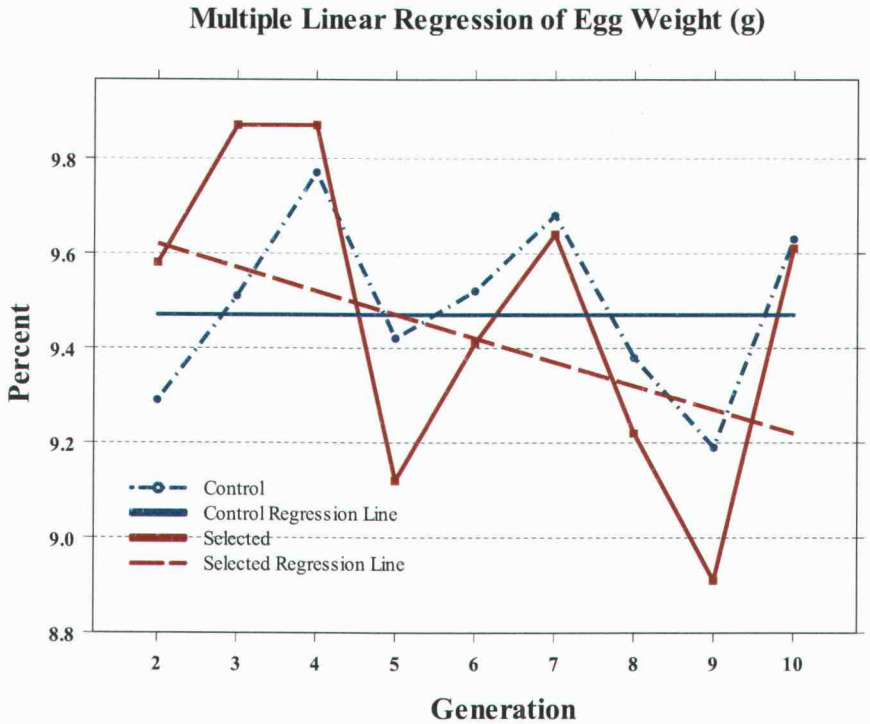

Figure 4.7 Multiple linear regression lines for mean egg weights. The residual standard error is 0.23. Multiple R^2=0.17. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 9.47 - 0.0005x$
(0.19) (0.03)
Line S: $y = 9.467 + 0.0004x$
(0.19, 0.25) (0.03, 0.04)

results of genic reorganization associated with selection, possibly suggestive of a small number of unknown loci responsible for hatchability traits. There also may be a treatment effect due to increased incubation temperature in Line S, or a gene-environment interaction.

Post-Hatch Mortality (0-10 days)

Post-hatch mortality of breeder candidate chicks was recorded and summarized in Figure 4.8 and Appendix Table A1.6. Calculation of the Bonferroni correction for family-wise variation yielded $\alpha = 0.005$. In the second generation, early post-hatch mortality between 0 and 10 days of age was significantly higher ($p<0.001$) in Line C than in Line S, by 19.1%. In generations 7, 9, and 10, mortality increased substantially in Line S ($p<0.001$ in generations 9 and 10, and $p<0.01$ in generation 7), with differences of 14.6% in generation 7, 4.9% in generation 27.5% in generation 9, and 17.7% in generation 10.

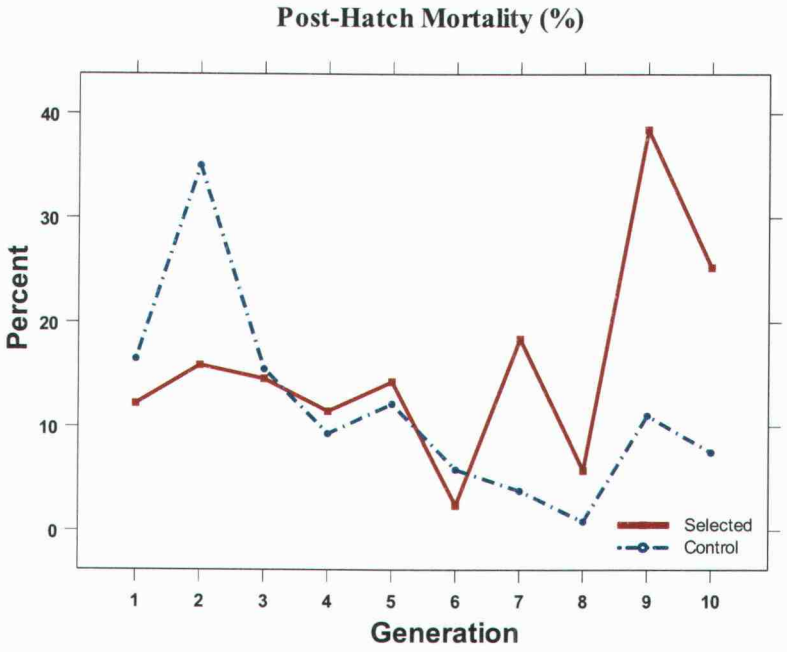

Figure 4.8 Comparison of percent post-hatch mortality (0-10 days of age) between Lines C and S over 10 generations of selection.

To compare performance between Lines C and S, MLR analysis was performed and summarized in Figure 4.9. The low *p*-value of 0.02 for the interaction coefficient provides strong evidence that an interaction exists between generations and lines for post-hatch mortality. A linear model may not be the best method of interpreting these data, due to the sharp fluctuations between generation intervals, but it provides a general indication of gains or losses due to selection over time. Post- hatch mortality has increased in Line S over the generations, while declining in Line C (Figure 4.9). It is possible that whatever genetic effect led to increased embryonic mortality may have also resulted in weaker Line S chicks.

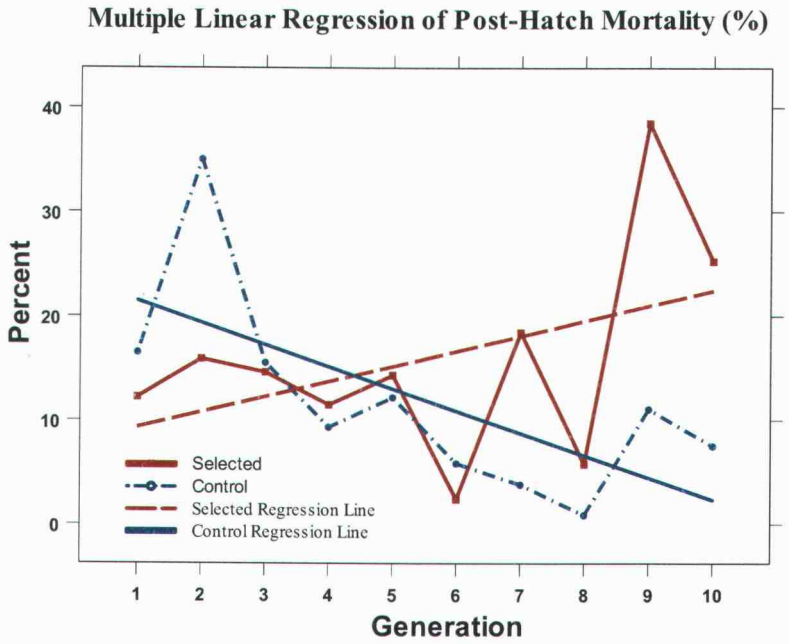

Figure 4.9 Multiple linear regression lines for post-hatch mortality. The residual standard error is 8.69. Multiple R^2=0.34. Standard errors for the correlation coefficients are included in parenthesis beneath the respective intercepts and slopes. The equations are:

Line C: $y = 23.24 - 2.09x$
(5.93) (0.96)
Line S: $y = 7.86 + 1.46x$
(5.93, 8.39) (0.96, 1.35)

Incubation Traits

The main goal of this research project was to examine the effect of selection for embryonic response to high temperature incubation, that is, to increase hatchability while reducing the incubation period. The incubation traits examined were fertility, the incidence of embryonic mortality during the three stages of incubation (D1, D2, and D3), incidence of pipped eggs, and hatchability of fertile eggs.

Calculation of the Bonferroni correction for family-wise variation yielded α = 0.005 for all of these traits.

Fertility

Fertility, measured during 10 generations of selection, is summarized in Figure 4.10 and Appendix Table A1.7. These results denote true fertility as opposed to candling fertility. Fertility was notably low in both lines in the first generation, which was attributed to advanced breeder age. Across the first five generations, fertility improved in Line S from 86.3% in generation 1 to 99.4% in generation 5. In subsequent generations, fertility fluctuated dramatically in Line S, from 84% in generation 7 and 83.5% in generation 9, to 96.1% and 91.5% in generations 8 and 10 respectively. There were significant differences in fertility between the lines in generations 3, 5, 7, and 9. ($p<0.001$). In the third generation, fertility was 4.7% higher in Line C than in Line S. In the next two generations, fertility was higher in Line S, by 3.7% in generation 4 ($p=0.052$) and by 5.2% in generation 5 ($p<0.0001$). Fertility was similar in both lines in generation 6, after which it declined considerably in Line S in the seventh generation, a difference of 14.4% between lines ($p<0.0001$). In the eighth generation, fertility increased substantially in Line S, for a difference of 1.8% between lines ($p=0.05$). In the ninth generation, Line S fertility dropped to its lowest point for a difference between lines of 9.8% ($p<0.0001$). In this generation, fertility also declined in Line C by 4.5%. In the tenth generation, fertility in Line S was still 3.2% lower than that of Line C ($p=0.02$). Due to the dramatic fluctuations observed in Line S, a linear regression

analysis was inappropriate to further analyze the results. It is unclear what could have caused these fluctuations, but it is possible that it was due in part to unknown environmental factors, or perhaps some kind of gene-environment interaction.

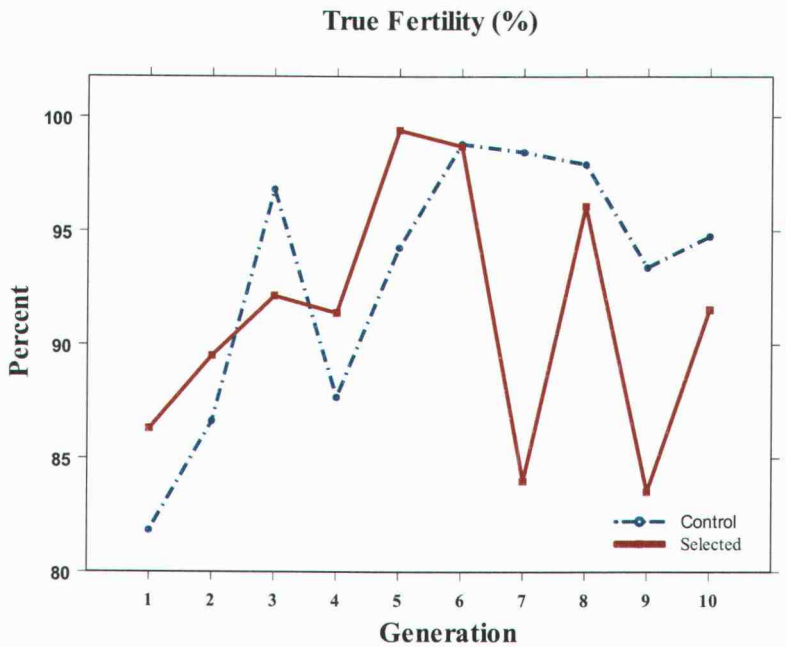

Figure 4.10 Comparison of percent true fertility between Lines C and S over 10 generations of selection.

Embryonic Mortality

Embryonic mortality in *Coturnix* has been observed to occur in a typical distribution, an example of which is illustrated in Figure 4.11. The majority of embryonic deaths occur during one of two periods—during the first three days of incubation (D1), and just prior to hatching (D3 and pipped embryos). There is also generally a slight mid-incubation (D2) peak (Woodard *et al.*, 1973).

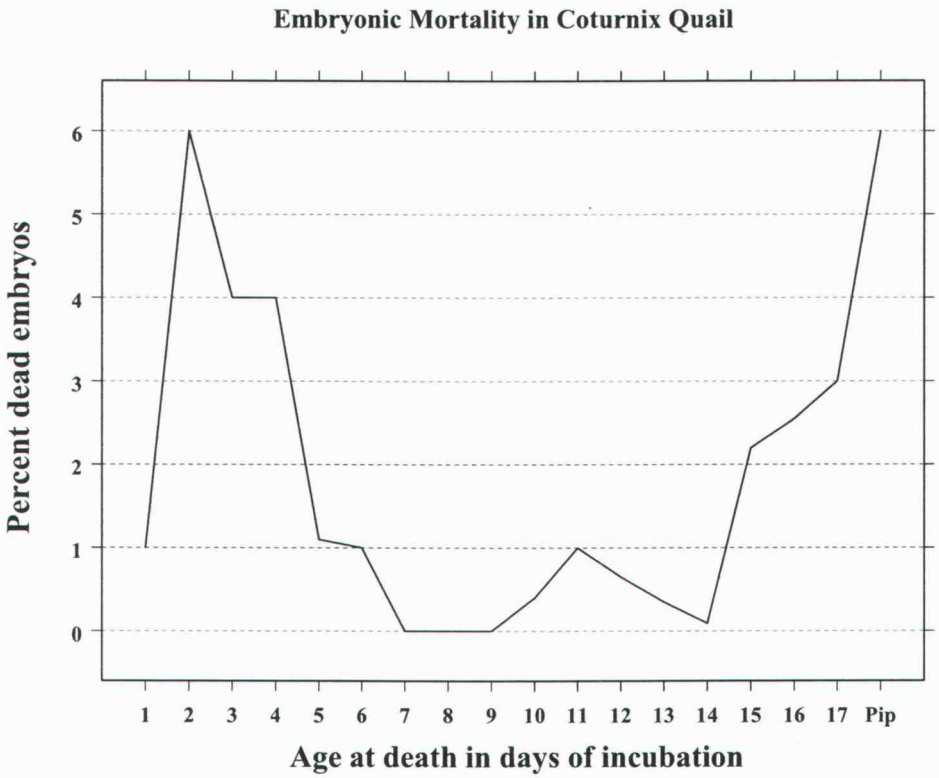

Figure 4.11 Embryonic mortality of *Coturnix* (adapted from Woodard *et al.*, 1973).

Incidence of Early Dead (D1) Embryos

The incidence of early embryonic mortality (D1, days 0-7 of incubation) was recorded for each generation and summarized in Figure 4.12 and Appendix Table A1.8. Incidence of D1 mortality was substantial (greater than 5 %) in both lines in the first generation, which was attributed to the advanced age of the breeder population. Line C exhibited a 7.8% higher incidence than Line S, however, this difference is not significant (p=0.08). This was somewhat unexpected, since it was anticipated that Line S D1 mortality would be greater than that of Line C.

Incubation at a higher temperature generally results in an increased early embryonic mortality (North and Bell, 1990). After the first generation,

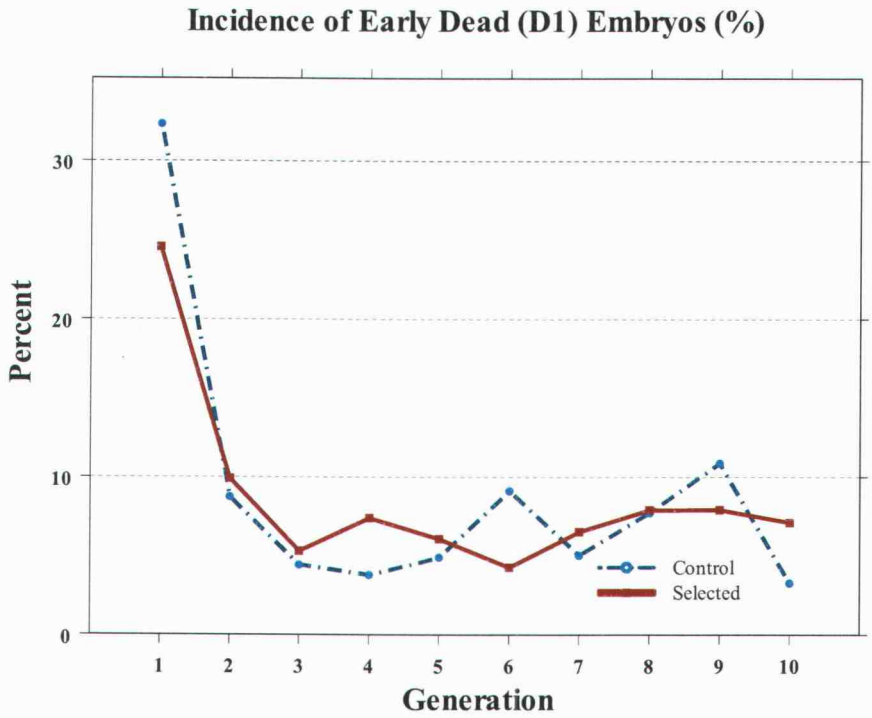

Figure 4.12 Comparison of the mean incidence (percent)of early dead (D1) embryonic mortality observed in Lines C and S over 10 generations of selection.

incidence of D1 embryos declined to a level below 10% in both lines, with the exception of generation 9, in which Line C sustained 10.9%. The only differences between lines occurred in generations 6 and 10. In generation 6, the incidence in Line C was 4.84% higher than Line S ($p<0.001$), and in generation 10 the incidence in Line S was 3.9% higher than Line C ($p=0.002$). Even when influential data points (Line C: generations 1, 9, and 10, and Line S: generations

1 and 2) are excluded, the remaining data are not well adapted to linear regression analysis (Multiple R^2= 0.097) so MLR results are not reported.

Incidence of Mid-Dead (D2) Embryos

The percent mid-incubation embryonic mortality (days 8-14 of incubation) was recorded for each generation and summarized in Figure 4.13 and Appendix Table A1.9. Again, as noted for the D1 mortality, D2 mortality in generation 1 was higher than expected under normal conditions (see Figure 4.11). These results may be attributed to the advanced age of the breeder population. Mortalities in Line C for generations 2 to 10 appear to have been at expected levels, except in the second

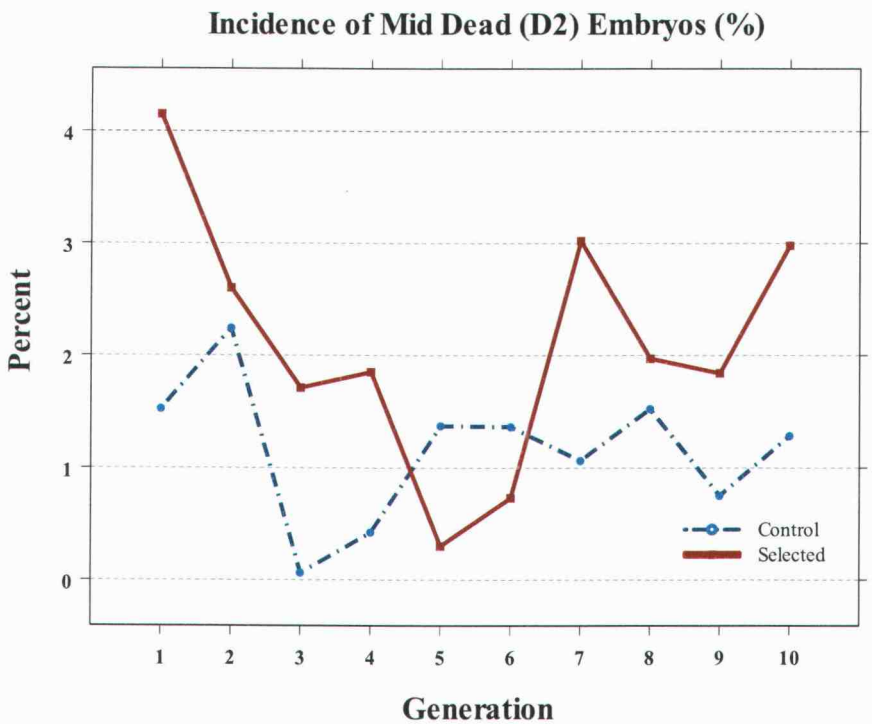

Figure 4.13 Comparison of the mean incidence (percent) of mid dead (D2) embryonic mortality observed in Lines C and S over 10 generations of selection.

generation. The incidence of D2 embryos was higher in Line S than in Line C, except in the fifth and sixth generations. There were no significant differences between lines.

To examine the effect of selection over time, MLR analysis was performed and summarized in Figure 4.14. Data for generation 1 were excluded due to the

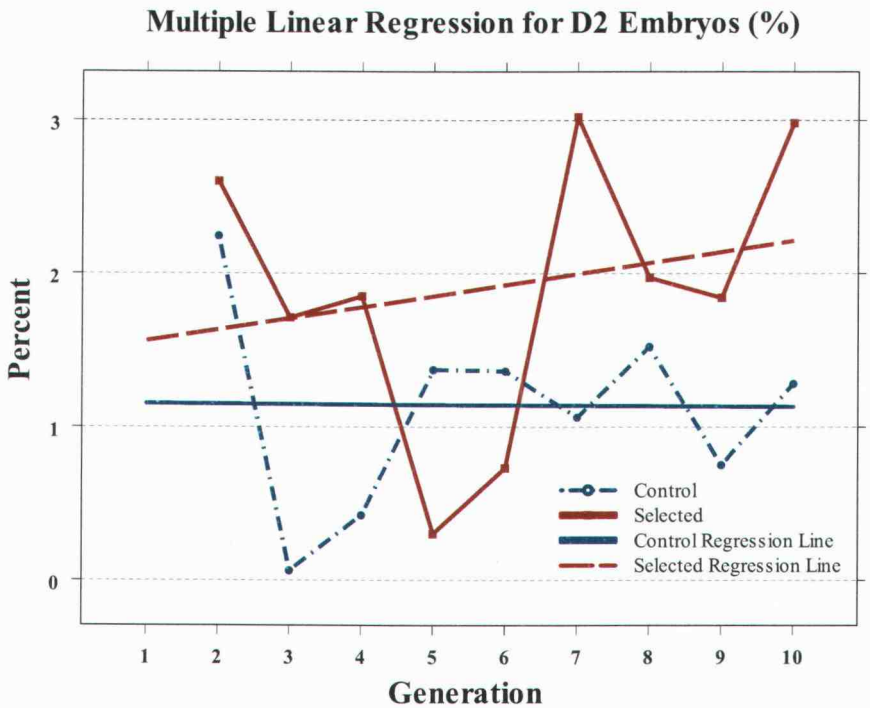

Figure 4.14 Multiple linear regression lines for the incidence of D2 embryos. The residual standard error is 0.9543. Multiple R^2=0.24. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 1.11 - 0.002x$
(0.71) (0.11)
Line S: $y = 1.40 - 0.081x$
(0.71, 0.74) (0.11, 0.17)

breeder age effect. An examination of Cook's Distance plots for these data indicated that generation 2 (both lines) influenced the slopes of the lines. Results of excluding these data points are summarized in Figure 4.15. With selection, the incidence of D2 embryos has increased in Line S (with the exception of generations 5 and 6), while remaining constant in Line C from generations 5

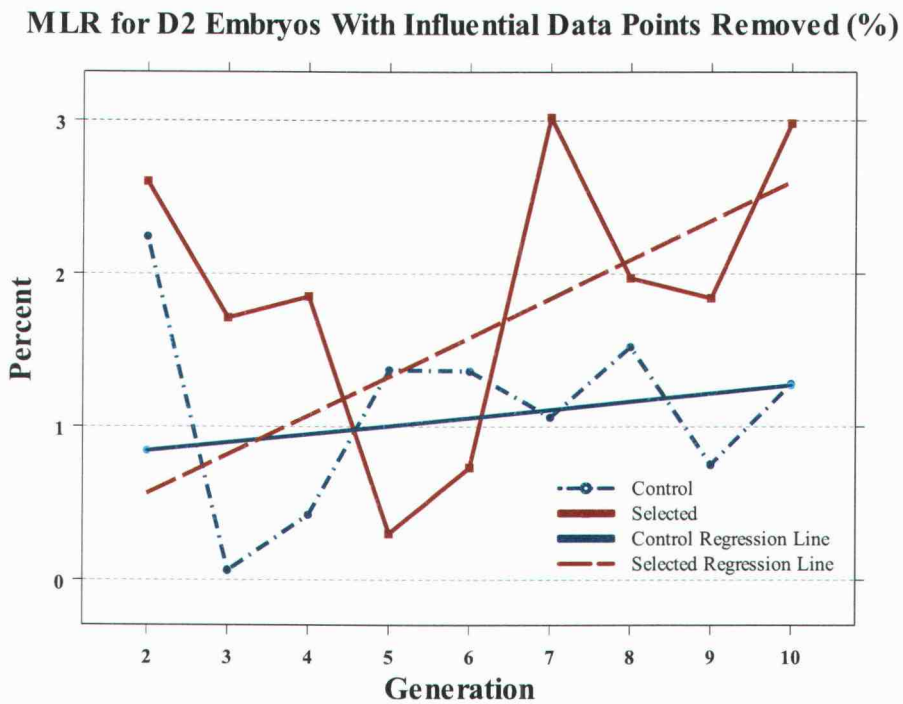

Figure 4.15 Multiple linear regression for the incidence of D2 embryos with influential data points removed: generation 2, both lines. The residual standard error is 0.73. Multiple R^2=0.41. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 0.73 + 0.054x$
(1.01) (0.05)
Line S: $y = 0.05 + 0.26x$
(1.01, 1.20) (0.05, 0.17)

through 10. The variation observed in Line S may be the result of an environmental component (possibly the high incubation temperature), but genic segregation may also have occurred.

Incidence of Late Dead (D3) Embryos

The percent embryonic mortality observed during the late incubation period (D3, days 15 to 17 of incubation) for each generation is summarized in Figure 4.16 and Appendix Table A1.10. The D3 mortality was exceptionally high in Line S in the first generation, which probably was the result of a treatment effect (with high temperature incubation resulting in increased mortality at this stage of development), since mortality was significantly lower in the control line ($p<0.0001$). If these results had been due to the age of the breeders, one would expect similar results in both lines. With the exception of generation 2, D3 mortality in Line C was observed at anticipated levels (Figure 4.11). The incidence of late dead embryos was higher in Line S as compared to Line C in all generations except generations 2 and 5, where the differences were not significant. Mortality in Line S in generations 2 to 6 was considerably reduced as compared to the first generation. This could be the result of successful genetic selection for physiological adaptation by the embryo to develop at the elevated incubation temperaature during the late incubation period. The substantial increase in mortality during subsequent generations may suggest that genic segregation had

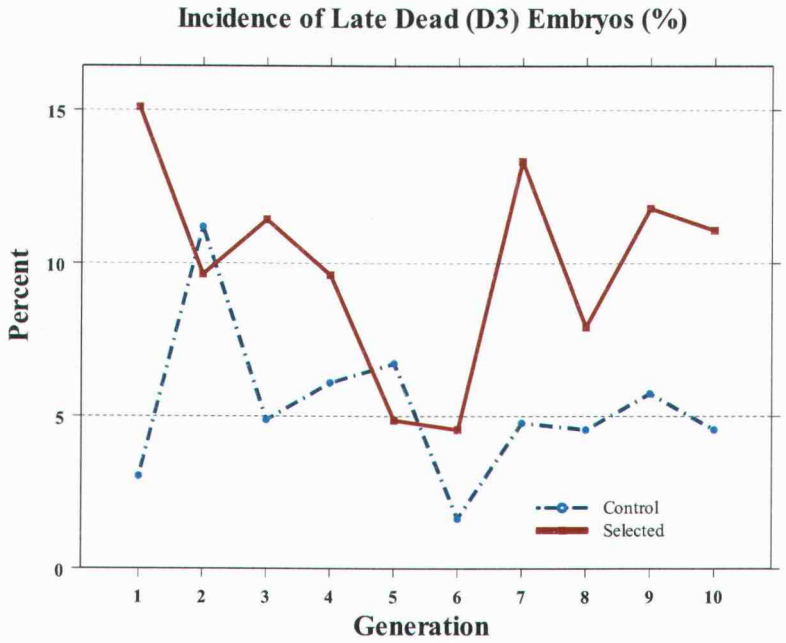

Figure 4.16 Comparison of mean incidence (percent) of late dead (D3) embryonic mortality observed in Lines C and S over 10 generations of selection.

occurred within Line S. In generations 3, 6, 7, 9, and 10, the differences between lines were statistically significant, ranging between 2.9% in generation 6 ($p<0.01$) to 12.1% in generation 1 ($p<0.001$; see Appendix table A1.10 for details).

An MLR analysis was performed in order to compare differences in mean incidence of D3 embryos between Lines C and S (Figure 4.17). Over time, Line S

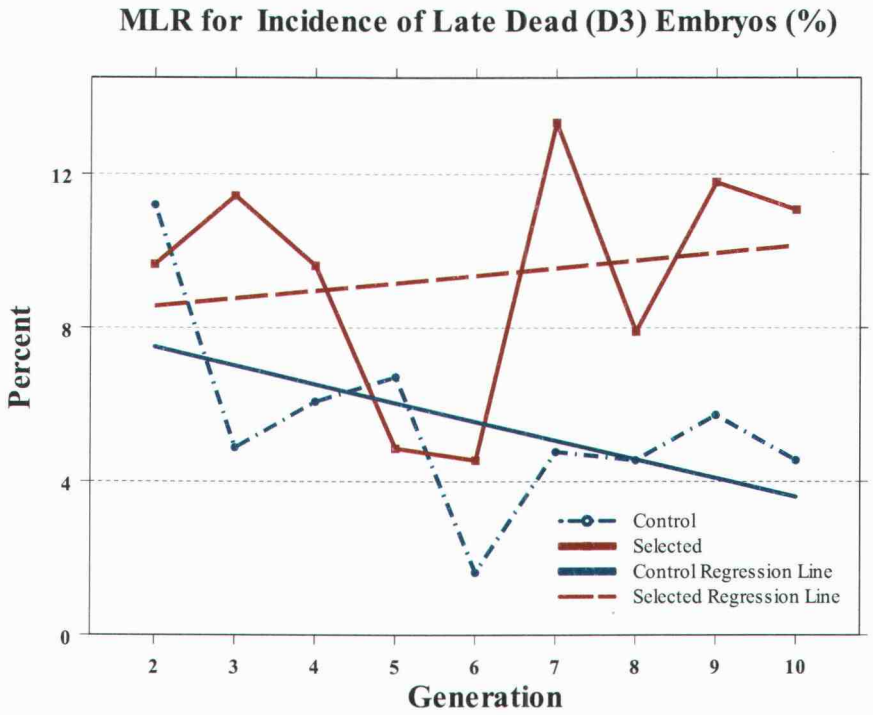

Figure 4.17 Multiple linear regression lines for incidence of D3 embryos. The residual standard error is 3.12. Multiple R^2=0.42. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 6.49 - 0.21x$
(2.36) (0.36)
Line S: $y = 10.86 - 0.17x$
(2.336, 3.34) (0.36, 0.51)

appears to have become more susceptible to D3 embryonic mortality, while Line C has become less susceptible. Again, genic segregation may have been occurring due to selection in Line S.

Incidence of Pipped Eggs

Those embryos that broke through the shell but were unable to complete the hatching process (pipped eggs) are summarized in Figure 4.18 and Appendix Table A1.11. The pipped egg incidence was low in the first generation. There were no significant differences between lines in the first five generations of the study. Significant line differences did not become apparent until the sixth generation, at which time Line S had an 8.5% incidence of pipped eggs, which was 4.7% higher than in Line C (p=0.004). In the seventh generation the incidence of pipped eggs declined slightly in Line S and substantially in Line C, with the incidence in Line S being 13.3%, giving a difference of 8.6% ($p<0.001$). In generation 8, Line S exhibited a dramatic increase in incidence of pipped eggs at 13.8%, the highest observation in all ten generations. There was also an increase in Line C, at 7.2%, for a difference of 6.6% ($p<0.001$). Between generations 7 and 10, both lines experienced either concurrent increases or decreases in mortality, suggesting a common environmental effect. Environmental factors that were in common at this stage of incubation included breeder age and management, as well as the hatcher environment. Thus the incidence of pipped eggs suggests a genotype x environment interaction such that Line S may require a different hatcher environment in order to minimize the incidence of pipped eggs. It is possible that the sudden decrease in temperature from 102 to 98.5 F is sufficiently stressful to increase the incidence of weak (*i.e.*, metabolically stressed) chicks in Line S. This would be an ideal area for future study. In the ninth generation, incidence of

pipped eggs declined in both lines; however, it remained higher in Line S for a difference of 3.4% ($p<0.05$). In the tenth generation incidence of pipped embryos declined slightly in Line S while increasing in Line C.

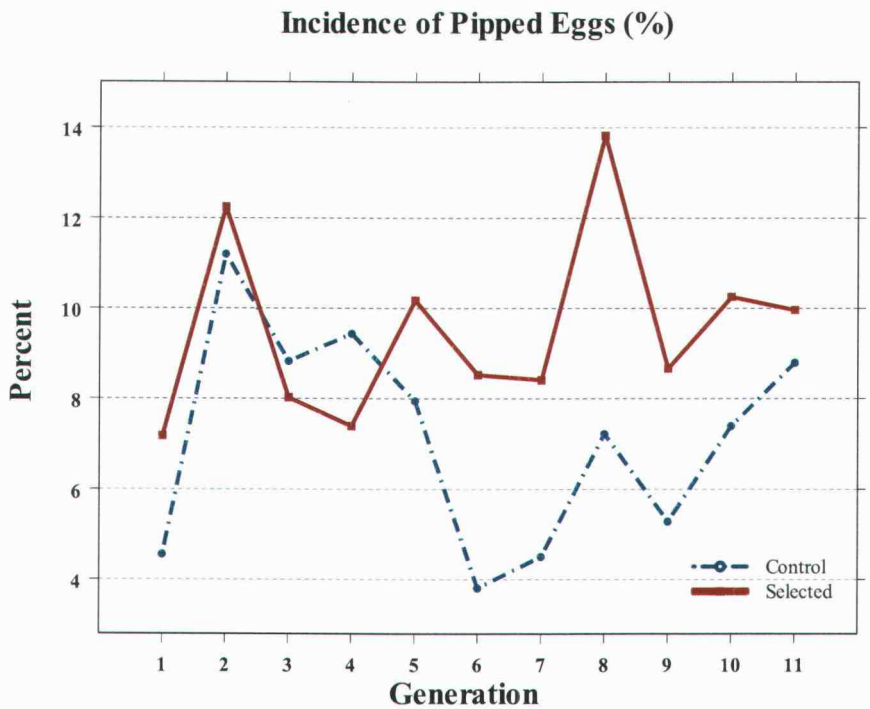

Figure 4.18 Comparison of the mean incidence (percent) of pipped eggs observed in Lines C and S over 10 generations of selection.

The MLR analysis performed to compare the mean incidence of pipped embryos between Lines C and S is presaented in Figure 4.19. Over all ten generations, the incidence of pipped embryos increased in Line S, while decreasing in Line C.

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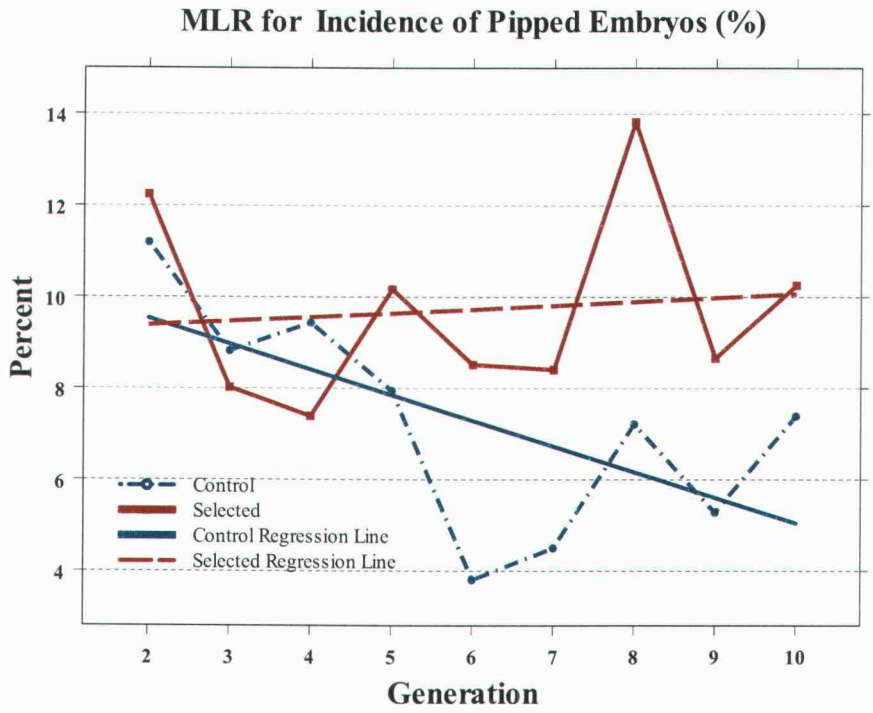

Figure 4.19 Multiple linear regression lines for incidence of pipped embryos. The residual standard error is 0.31. Multiple R^2=0.42. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 10.65 - 0.56x$
(1.80) (0.27)
Line S: $y = 9.21 + 0.22x$
(1.80, 2.54) (0.27, 0.39)

Hatchability of Fertile Eggs

Mean hatchability of fertile eggs data from each generation are summarized in Figure 4.20 and Appendix Table A1.12. Hatchability of fertile eggs was consistently higher in Line C than in Line S, with the exception of generations 2 and 5. There were significant differences between lines in generations 3, 4, 7, 8, 9, and 10 (see Appendix Table A1.12 for *p*-values). Hatchability in both lines was at

its lowest in the first generation, where Line S was at 48.7% and Line C at 57.6%, a difference of 8.9% (p=0.07). Again, this low incubation trait value can be

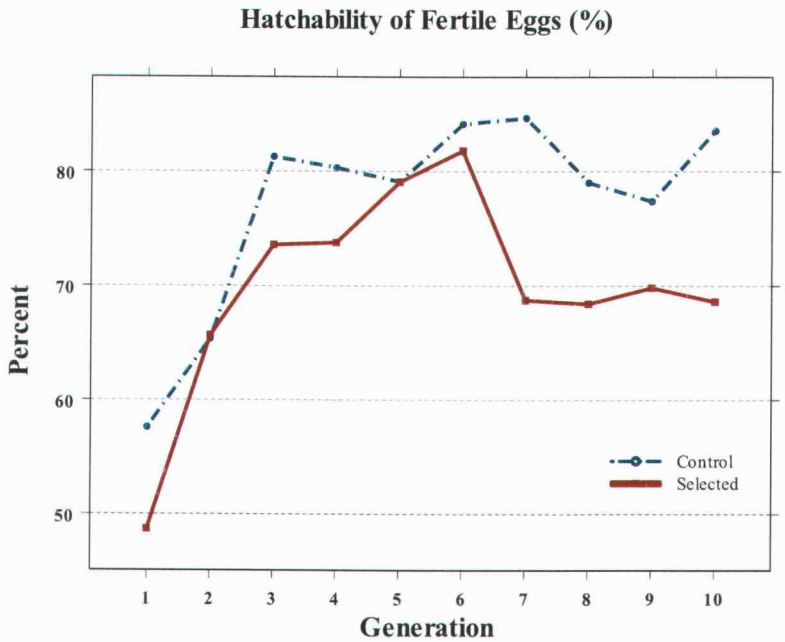

Figure 4.20 Comparison of mean percent hatchability of fertile eggs observed in Lines C and S over 10 generations of selection.

attributed to the advanced age of the breeder population. Hatchability improved in Line S over the first six generations, reaching a peak of 81.8%, after which it declined in subsequent generations to 68.6% in generation 10.

An MLR analysis was performed in order to compare mean performance between lines C and S over the generations of selection (Figure 4.21). Examination of Cook's Distance plots indicated that the generation 2 data (which were quite low compared to subsequent generations) were highly influential, so MLR results with

these data excluded are presented in Figure 4.22. With selection, there has been a decrease in hatchability in Line S, while hatchability was virtually unchanged

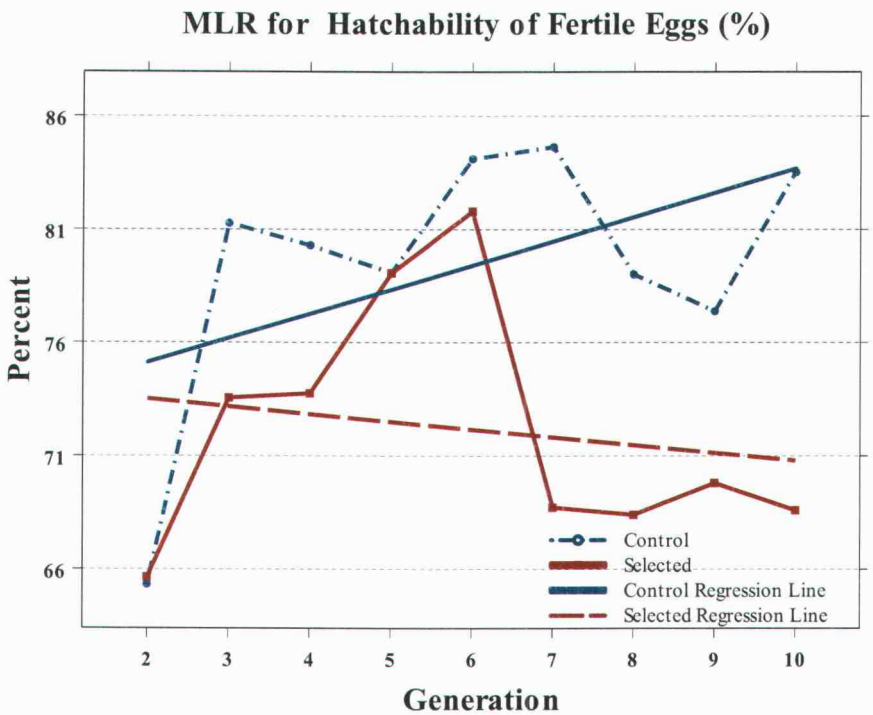

Figure 4.21 Multiple linear regression lines for percent hatchability of fertile eggs. The residual standard error is 7.99. Multiple R^2= 0.42. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: y = 66.39 + 1.97x
(4.67) (0.71)
Line S: y = 64.12 + 1.03x
(4.67, 6.60) (0.71, 1.01)

in Line C if generation 2 is removed. These results suggest that selection may have resulted in a plateau of additive genetic effects. It is generally expected that the greatest response to selection will be observed during the initial stages of selection, followed by a gradual decline (Marks, 1985).

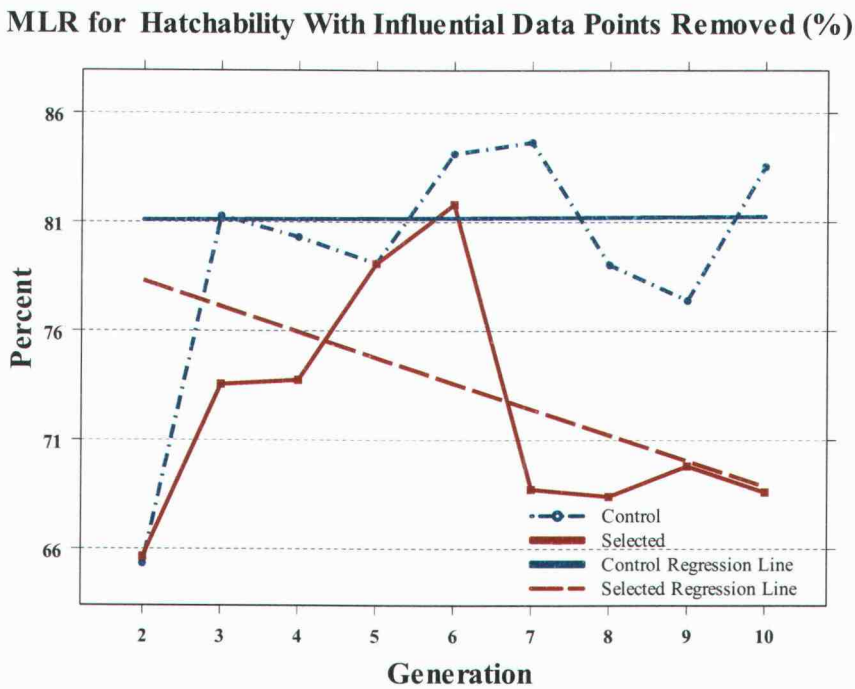

Figure 4.22 Multiple linear regression for percent hatchability of fertile eggs with influential data points removed: generation 2, both lines. The residual standard error is 3.82. Multiple R^2=0.65. Standard errors for the correlation coefficients are included in parentheses beneath the respective intercepts and slopes. The equations are:

Line C: $y = 81.04 + 0.02x$
(4.06) (0.59)
Line S: $y = 80.66 - 1.18x$
(4.06, 5.75) (0.59, 0.83)

Parthenogenesis

Examination of incubated infertile eggs from virgin hens of Lines C and S in generations 2, 3, 4, 6, 7, and 9 were inconsistent in the detection of parthenogenetic development across the generations. In generations 2, 6, and 9 no development was detected, while in generations 3, 4, and 7 a total of 10 parthenogens was detected in 1,133 eggs examined. It appears that parthenogenetic

expression in *Coturnix,* consisting mainly of unorganized membranes, is not as extensive or varied as has been observed in turkeys. Due to the absence of repeatable occurrences, no further studies were conducted.

Conclusion

When genetic selection was applied to the incubation environment of fertile Japanese quail eggs at 102 F versus the normal temperature of 100 F, body weight was consistently elevated in both sexes in Line S. Sexual maturity "trended" to occur earlier in Line S, although it was the same as Line C in the final two generations. Hen-day egg production lower in Line S for the last five generations, while egg weight was generally higher in Line C. Post-hatch mortality increased in Line S over the latter generations. Although Line S fertility fluctuated widely from generation to generation, it was lower than in Line C. The incidence of embryonic mortality generally was higher in Line S, with a concurrent reduction in hatchability. It is possible that many of these traits (such as body weight) were negatively correlated to fitness traits (such as hen-day egg production, fertility, and hatchability). In past reported studies, negative relationships have been observed between high body weight and egg production, fertility, hatchability, and sexual maturity (Marks, 1985). In general, egg production may be influenced by any factor affecting sexual development (Shanaway, 1994). Negative genetic correlations have been observed in Japanese quail between egg production and body weight, egg weight and egg production, and possibly between body weight and egg weight (Marks, 1990).

Responses fluctuated for most traits from generation to generation, which is commonly experienced in genetic selection studies. Scossiroli (1953), for example,

observed such fluctuations in the number of sternopleural hairs in *Drosophila melanogaster*. Progress of the selected trait (number of hairs) was attained in successive steps that were alternately positive and negative. These fluctuations were attributed to an "over-response" to artificial selection pressure, which results in an unbalanced condition that is remedied by a swing in the opposite direction in the following generation. No mechanism was postulated for this phenomenon, but it could be related to Lerner's genetic homeostasis theory, in which artificial and natural selection are at odds with each other in regards to fitness traits.

Analyses of the data for several traits (*i.e.*, fertility, D2, and hatchability) by MLR suggest that the observed results for both lines in generations 1 and 2 may have been spurious. It is possible that reorganization of the genome occurred in the first two generations, which may have been a function of the animal, the trait in question, or due to some unspecified environmental factor (such as breeder age or management practices). Because of this, it may be justified to disregard the first two generations in the assessment of genetic improvement over the generations.

Since any factor influencing increased embryonic mortality concurrently reduces hatchability, viewing hatchability as a single trait is over-simplistic. Hatchability is in actuality the absence of any number of embryonic lethal genes and/or gene-environment interactions. For optimum hatchability, a polygenic trait, it is necessary to have a "stabilized and harmonious gene complex" (Dubinin, quoted in Lerner, 1954. An increase in homozygosity through artificial selection may destabilize the complex and disrupt its harmony, leading to possible lethal effects.

It is possible that overdominance, in which the heterozygote is more fit (has better survivability) than either homozygote, plays a role in hatchability traits. An increase in homozygosity could increase the segregational load, thus reducing fitness traits such as fertility and hatchability.

While selection for hatchability alone has been successful in past reported studies, addition of an environmental factor known to increase embryonic mortality, *i.e.*, increased incubation temperature, appears to have had unpredicted results. In order to fully explain this phenomenon, it would be necessary to ascertain the physiological effects of high temperature incubation in both Lines C and S. It is difficult to determine to what extent the observed results in Line S are due to treatment effect, to genetic selection, or to gene-environment interactions. One way to determine these factors would be to do reciprocal settings for each generation (see Appendix 3). Chicks from each of the four treatment/line combinations would need to be reared and allowed to reproduce in order to determine if Line S exhibits a higher growth rate even when incubated at normal temperature, and/or matures earlier, etc. It might also be of interest to determine chicks' body weights at hatch and again at four weeks of age to determine growth rate, and what correlations, if any, exist between growth rate and other traits of interest such as onset of sexual maturity. It is possible that selection for high hatchability at an elevated incubation temperature is in some way an inadvertent selection for increased body weight, which would provide an explanation for the results observed in this experiment.

Based upon observations over 10 generations of selection, it seems likely that genic segregation occurred within Line S, resulting in declines in reproductive traits (such as egg production, fertility, and hatchability) and therefore fitness. Although embryonic development time was effectively reduced by about one day, it seems unlikely that this benefit can compensate for the observed reductions in hatchability and other fitness traits. It is possible that relaxing selection pressure for a few generations might permit a return of altered genetic variability to be attained, after which selection could be reinitiated, but perhaps at a less intense rate.

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Appendices

Appendix 1

Tables of Data

The following tables are designed to provide a synopsis of the results of this genetic selection study, conducted over 10 generations of selection. Included are the summaries of male body weights, female body weights, onset of female sexual maturity, hen-day egg production, egg weights, post-hatch mortality, fertility, early dead (D1) embryonic mortality, mid dead (D2) embryonic mortality, late dead (D3) embryonic mortality, incidence of pipped eggs, and hatchability of fertile eggs.

Appendix 1

Table A1.1 Mean body weights in grams and standard errors (SE) of male Japanese quail of Lines C and S in generations 1-10 (birds were not weighed in generations 7, 8, and 9). The number of birds weighed is denoted by "n" for each line. The *p*-values* are derived from *t*-tests for the difference of means within generations.

Gen.	Age (weeks)	Line C (g)	n	Line C SE	Line S (g)	n	Line S SE	*p*-Value
1	5.0	102.9	26	4.99	103.6	30	5.46	0.6295
2	4.7	84.4	53	7.56	90.1	50	7.67	0.0003
3	4.0	82.7	62	6.72	88.2	54	6.99	<.0001
4	5.3	102.2	34	6.11	110.5	43	8.18	<.0001
5	4.0	92.0	57	5.82	96.3	71	5.61	<.0001
6	4.9	100.7	120	6.27	105.7	78	6.01	<.0001
10	4.6	90.2	93	6.61	103.4	34	7.23	<.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.2 Mean body weights in grams and standard errors (SE) of female Japanese quail of Lines C and S in generations 1-10 (birds were not weighed in generations 7, 8, and 9). The number of birds weighed is denoted by "n" for each line. The *p*-values* are derived from *t*-tests for the difference of means within generations.

Gen.	Age (weeks)	Line C (g)	n	Line C SE	Line S (g)	n	Line S SE	*p*-Value
1	5.0	105.4	49	5.55	111.3	35	9.49	0.0006
2	4.7	86.8	66	6.76	92.6	57	8.73	0.0001
3	4.0	85.5	71	7.61	92.6	65	7.50	<.0001
4	5.3	109.7	47	6.41	114.4	40	7.15	0.0018
5	4.0	95.1	55	5.07	99.1	69	6.72	0.0004
6	4.9	105.3	89	5.67	111	78	8.35	<.0001
10	4.6	95.6	88	7.78	107.4	42	6.25	<.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.3 Average hen age at onset of sexual maturity (age at first egg) in days and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of hens is denoted by "n" for each line. The *p*-values* are derived from *t*-tests for the difference of means within generations.

Gen.	Line C (days)	n	Line C SE	Line S (days)	n	Line S SE	*p*-Value
1	53.5	26	8.13	55.0	23	7.94	0.5012
2	59.8	25	2.96	58.2	25	6.03	0.2282
3	57.0	25	6.20	51.3	25	3.80	0.0004
4	63.6	24	6.17	60.9	25	7.35	0.1611
5	72.0	24	4.94	65.0	25	11.32	0.0081
6	55.9	25	4.73	54.1	25	9.08	0.3941
7	60.7	25	3.41	56.3	25	6.52	0.0044
8	57.1	25	2.84	53.7	24	4.55	0.0025
9	61.7	25	8.43	61.4	25	7.47	0.8800
10	57.6	25	4.97	57.9	25	8.18	0.8679

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.4 Mean percent hen-day egg production and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The *p*-values* are derived from tests for difference in proportions within generations.

Generation	Line C (%)	Line C SE	Line S (%)	Line S SE	*p*-Value
1	83.24	0.013	82.87	0.013	0.8864
2	85.18	0.010	83.97	0.010	0.4234
3	78.86	0.011	82.00	0.011	0.0507
4	88.61	0.009	87.45	0.009	0.4056
5	90.92	0.008	84.46	0.010	<.0001
6	92.26	0.007	84.87	0.009	<.0001
7	95.08	0.005	89.72	0.007	<.0001
8	89.21	0.008	84.84	0.010	0.0005
9	91.13	0.007	83.09	0.010	<.0001
10	83.51	0.008	74.01	0.009	<.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.5 Mean egg weights in grams and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of eggs weighed is denoted by "n" for each line. The *p*-values* are derived from *t*-tests for the difference of means within generations.

Gen.	Line C (g)	n	Line C SE	Line S (g)	n	Line S SE	*p*-Value
1	10.3	377	0.19	10.3	377	0.19	NA
2	9.29	253	0.11	9.58	224	0.02	0.0696
3	9.51	676	0.11	9.87	651	0.02	0.0007
4	9.67	560	0.31	9.53	622	0.31	0.1890
5	9.42	710	0.40	9.12	676	0.24	0.0060
6	9.52	766	0.24	9.41	699	0.37	0.2479
7	9.61	799	0.24	9.54	757	0.27	0.3606
8	9.39	824	0.25	9.22	769	0.17	0.0132
9	9.19	725	0.46	8.91	520	0.37	0.0064
10	9.63	756	0.35	9.61	669	0.60	0.8593

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.6 Mean percent post-hatch mortality (0-10 days) and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The total number of hatched chicks is denoted as "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

Gen.	Line C (%)	n	Line C SE	Line S (%)	n	Line S SE	*p*-Value
1	16.5	97	0.11	12.2	123	0.03	0.4746
2	35.0	100	0.15	15.9	107	0.04	0.0026
3	15.5	155	0.10	14.6	144	0.03	0.9555
4	9.3	86	0.09	11.5	96	0.03	0.8165
5	12.1	107	0.11	14.3	77	0.03	0.8395
6	5.9	222	0.07	2.4	166	0.01	0.1645
7	3.8	131	0.06	18.4	76	0.03	0.0011
8	0.9	230	0.03	5.8	190	0.02	0.009
9	11.0	136	0.10	38.5	117	0.04	<.0001
10	7.5	200	0.08	25.2	107	0.03	<.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.7 Mean fertility (%) and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The total number of eggs set is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

Gen.	Line C (%)	n	Line C SE	Line S (%)	n	Line S SE	*p*-Value
1	81.82	242	0.025	86.32	307	0.020	0.1861
2	86.63	516	0.015	89.51	429	0.015	0.2097
3	96.8	656	0.007	92.14	636	0.011	0.0004
4	87.68	544	0.014	91.39	592	0.012	0.0517
5	94.24	695	0.009	99.40	663	0.003	<.0001
6	98.79	745	0.004	98.70	690	0.004	0.8699
7	98.44	767	0.004	84.00	750	0.013	<.0001
8	97.9	808	0.005	96.07	738	0.007	0.0500
9	93.38	710	0.009	83.54	650	0.015	<.0001
10	94.75	743	0.008	91.53	661	0.011	0.0218

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.8 Mean percent early dead (D1) embryos and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of fertile eggs is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

Gen.	Line C (%)	n	Line C SE	Line S (%)	n	Line S SE	*p*-Value
1	32.32	198	0.033	24.53	265	0.026	0.0808
2	8.72	447	0.023	9.90	384	0.015	0.6452
3	4.41	635	0.007	5.29	586	0.011	0.5597
4	3.77	477	0.015	7.39	541	0.012	0.0187
5	4.89	655	0.009	6.07	659	0.003	0.4111
6	9.1	736	0.004	4.26	681	0.004	0.0004
7	5.03	755	0.004	6.51	630	0.013	0.2882
8	7.71	791	0.005	7.90	709	0.007	0.9695
9	10.86	663	0.009	7.92	543	0.015	0.1028
10	3.27	704	0.008	7.11	605	0.011	0.0024

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1. 9 Mean percent mid dead (D2) embryos and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of fertile eggs is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

	Line C			Line S			
Gen.	**(%)**	**n**	**Line C SE**	**(%)**	**n**	**Line S SE**	***p*-Value**
1	1.52	198	0.009	4.15	265	0.012	0.1725
2	2.24	447	0.007	2.6	384	0.008	0.9067
3	0.06	635	0.003	1.71	586	0.005	0.1346
4	0.42	477	0.003	1.85	541	0.006	0.0692
5	1.37	655	0.005	0.3	659	0.002	0.0677
6	1.36	736	0.004	0.73	681	0.003	0.3746
7	1.06	755	0.004	3.02	630	0.007	0.0152
8	1.52	791	0.004	1.97	709	0.005	0.6314
9	0.75	663	0.003	1.84	543	0.006	0.1515
10	1.28	704	0.004	2.98	605	0.007	0.0502

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.10 Mean percent late dead (D3) embryos and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of fertile eggs is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

	Line C			Line S			
Gen.	**(%)**	**n**	**Line C SE**	**(%)**	**n**	**Line S SE**	***p*-Value**
1	3.03	198	0.012	15.09	265	0.022	<0.0001
2	11.19	447	0.015	9.64	384	0.015	0.5391
3	4.88	635	0.009	11.43	586	0.013	<0.0001
4	6.08	477	0.011	9.61	541	0.013	0.0498
5	6.72	655	0.010	4.86	659	0.008	0.1844
6	1.63	736	0.005	4.55	681	0.008	0.0023
7	4.77	755	0.008	13.33	630	0.014	<0.0001
8	4.55	791	0.007	7.90	709	0.010	0.0096
9	5.73	663	0.009	11.79	543	0.014	0.0003
10	4.55	704	0.008	11.07	605	0.013	<0.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.11 Mean percent pipped eggs and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of fertile eggs is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

Gen.	Line C (%)	n	Line C SE	Line S (%)	n	Line S SE	*p*-Value
1	3.03	198	0.012	15.09	265	0.022	0.3296
2	11.19	447	0.015	9.64	384	0.015	0.7163
3	4.88	635	0.009	11.43	586	0.013	0.6903
4	6.08	477	0.011	9.61	541	0.013	0.2888
5	6.72	655	0.010	4.86	659	0.008	0.1899
6	1.63	736	0.005	4.55	681	0.008	0.0003
7	4.77	755	0.008	13.33	630	0.014	0.0040
8	4.55	791	0.007	7.90	709	0.010	<0.0001
9	5.73	663	0.009	11.79	543	0.014	0.0276
10	4.55	704	0.008	11.07	605	0.013	0.0832

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Table A1.12 Mean percent hatchability of fertile eggs and standard errors (SE) for Japanese quail of Lines C and S in generations 1-10. The number of fertile eggs is denoted by "n" for each line. The *p*-values* are derived from tests for difference in proportions within generations.

Gen.	Line C (%)	n	Line C SE	Line S (%)	n	Line S SE	*p*-Value
1	57.58	198	0.035	48.68	265	0.031	0.0715
2	65.32	447	0.023	65.63	384	0.024	0.9859
3	81.26	635	0.015	73.55	586	0.018	0.0016
4	80.29	477	0.018	73.75	541	0.019	0.0167
5	79.08	655	0.016	79.06	659	0.016	0.9912
6	84.10	736	0.013	81.79	681	0.015	0.2773
7	84.63	755	0.013	68.73	630	0.018	<0.0001
8	79.01	791	0.014	68.4	709	0.017	<0.0001
9	77.38	663	0.016	69.8	543	0.020	0.0035
10	83.52	704	0.014	68.6	605	0.019	<0.0001

*The *p*-value indicates the significance of the difference between lines at α=0.005.

Appendix 2

Hatch Dates For Each Generation

Dates upon which chicks of each generation were removed from the incubator are included in the following table:

Generation	Hatch Date
1	July 16, 2001
2	October 5, 2001
3	January 17, 2002
4	April 10, 2002
5	July 18, 2002
6	October 31, 2002
7	February 7, 2003
8	May 23, 2003
9	August 21, 2003
10	December 11, 2004

Appendix 3

Reciprocal Settings

The following data were collected during the selection experiment but not presented in the thesis proper. This information is provided since it may have benefits to future investigations.

In order to determine how eggs from each line would perform at different incubation temperatures, in generations 4 and 6, eggs from each line (C and S) were randomly assorted into two groups per line, and one group from each line was incubated at each incubation temperature (100 F and 102 F). This provided a total of four line-temperature treatment combinations: Line C eggs incubated at 100 F, Line C eggs incubated at 102 F, Line S eggs incubated at 100 F, and Line S eggs incubated at 102 F. This procedure was performed for two consecutive weeks in each of the two generations. Tests of differences of proportions were performed to determine the existence of differences between treatment groups. The data and results of these experiments are summarized in Tables A3.1-A3.4.

When comparing eggs from Lines C and S incubated at 100 F (Table A3.1), in generation 4 there were significant reductions in Line S fertility ($p < 0.05$), increased incidence of D3 embryos ($p < 0.05$), and decreased hatchability ($p < 0.02$). There were no significant differences in generation 6.

In the comparison of eggs from each line incubated at 102 F, there were no significant differences between lines in generations 4 and 6 (Table A3.2). This could indicate that selection for improved hatchability at 102 F was not successful. Without data from all 10 generations of this study, however, it is difficult to determine whether this was the case in all generations.

When Line C eggs incubated at each temperature are compared (Table A3.3), there is a significant reduction in hatchability in the eggs incubated at 102 F in generation 4 ($p < 0.03$), and in incidences of D3 ($p < 0.008$) and pipped embryos ($p < 0.02$), as well as hatchability ($p = 0.0001$) in generation 6. These results were not unexpected, as embryonic mortality is normally higher at this temperature.

When comparing Line S embryos incubated at each temperature (Table A3.4), there were no significant differences in generation 4. In generation 6, incidence of pipped embryos was higher at 102 F ($p = 0.0002$), and hatchability was lower at the same temperature ($p = 0.0002$). This could indicate that over time Line S has lost its ability to hatch as well at 102 F, possibly due to additive genetic effects due to selection.

Table A3.1 Generation 4—comparison of eggs from each line (C and S) incubated at 100 F and 102 F. Two-sided *p*-values were generated from tests of differences in proportions.

Comparison of Eggs From Each Line Incubated at 100 F

Line	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	110	91.82	5.94	0.00	1.98	2.97	89.11
Selected	118	98.31	3.45	0.86	10.34	10.34	75.00
	***p*-Value:**	0.0483	0.58	0.35	0.03	0.06	0.01

Comparison of Eggs From Each Line Incubated at 102 F

Line	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	122	92.62	7.08	0.00	8.85	6.19	76.11
Selected	118	97.46	8.70	0.87	6.96	7.83	73.91
	***p*-Value:**	0.16	0.84	0.32	0.78	0.82	0.82

Table A3.2 Generation 4—comparison of eggs from Line C incubated at 100 F and 102 F, and eggs from Line S incubated at 100 F and 102 F. Two-sided *p*-values were generated from tests of differences in proportions.

Comparison of Control Eggs Incubated at Each Temperature

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	100F	110	91.82	5.94	0.00	1.98	2.97	89.11
Control	102F	122	92.62	7.08	0.00	8.85	6.19	76.11
		***p*-Value:**	0.82	0.95	NA	0.06	0.43	0.02

Comparison of Selected Eggs Incubated at Each Temperature

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Selected	100 F	118	98.31	3.45	0.86	10.34	10.34	75.00
Selected	102 F	118	97.46	8.70	0.87	6.96	7.83	73.91
		***p*-Value:**	1.0	0.16	1.0	0.50	0.66	0.97

Table A3.3 Generation 6—comparison of eggs from each line (C and S) incubated at 100 F and 102 F. Two-sided *p*-values were generated from tests of differences in proportions

Comparison of Eggs From Each Line Incubated at 100 F

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	100 F	141	99.29	7.86	0.00	1.43	2.14	89.29
Selected	100 F	120	100.00	8.33	0.00	4.17	0.83	88.67
		***p*-Value:**	0.36	0.89	NA	0.33	0.73	0.65

Comparison of Eggs From Each Line Incubated at 102 F

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	102 F	140	79.90	7.19	2.16	9.35	10.07	69.06
Selected	102 F	126	99.21	6.40	3.20	9.60	14.40	65.60
		***p*-Value:**	0.94	0.99	0.89	0.95	0.38	0.64

Table A3.4 Generation 6—comparison of eggs from Line C incubated at 100 F and 102 F, and eggs from Line S incubated at 100 F and 102 F. Two-sided *p*-values were generated from tests of differences in proportions.

Comparison of Control Eggs Incubated at Temperatures of 100 and 102 F

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Control	100F	141	99.29	7.86	0.00	1.43	2.14	89.29
Control	102F	140	99.29	7.19	2.16	9.35	10.07	69.06
		***p*-Value:**	1.0	0.83	0.24	0.01	0.01	0.0001

Comparison of Selected Eggs Incubated at Temperatures of 100 and 102 F

Line	Temperature	No. Eggs	Fertility (%)	D1 (%)	D2 (%)	D3 (%)	Pipped (%)	Hatchability (%)
Selected	100 F	120	100.00	8.33	0.00	4.17	0.83	86.67
Selected	102 F	126	99.21	6.40	3.20	9.60	14.40	65.60
		***p*-Value:**	0.33	0.74	0.14	0.16	0.0002	0.0002

Appendix 4

In the course of a genetic selection study for hatchability of Japanese quail embryos incubated at 102 F, an embryonic lethal condition was discovered and studied. Results were presented at the 92nd annual meeting of the Poultry Science Association in July of 2003. The abstract of this presentation (Poultry Sci. 82[Suppl. 1]:23-24) is reproduced below:

Ring Lethal-2, an Autosomal and Recessive Early Embryonic Failure in *Coturnix* Quail. T.F. Savage, W.R. Colvin*, and J.C. Hermes, *OR State University, Corvallis, OR.*

An early embryonic disorder affecting blastoderm development in *Coturnix* quail embryos (Poultry Sci. 80[Suppl. 1]:35, 2002) has been observed and studied. Ring Lethal-two (RL-2) describes an embryonic failure that was initially observed at 7 days of incubation and can be detected by 72 h. The disorder is characterized by the presence of an irregular shaped ring of blastoderm cells with a central mass of additional undifferentiated cells. The diameter of the ring and central cell mass have ranged from 3 to 13 mm and 1 to 4 mm, respectively. Expressivity of the disorder has varied from the presence of a blastoderm visible as a central cell mass to its presence encircled by a ring of dense cells approximately 1 mm in dia. Macroscopic studies have revealed that the disorder can be detected as early as 19 h of incubation. This condition, RL-2, has a resemblance to a previously described early embryonic failure in turkey embryos, Ring lethal (J Hered 76:474, 1985). This new disorder is not influenced by the length of pre-incubation storage, incubation temperature and duration. The mode of genetic transmission determined by select matings indicated the disorder was the expression of an autosomal and recessive gene. The gene locus and allele symbol, RL-2 is proposed for this embryonic developmental mutation.

Key Words: *Coturnix* quail, Embryo, Lethal mutation, Autosome, Recessive