Consequences of selection for fear in Japanese quail

Auswirkungen der Selektion auf Furcht bei der Japanischen Wachtel

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Introduction

Fear is a motivational system that protects animals from danger, particularly against predation. The expression of fear reactions is present in all animals and has an obvious adaption value. Given the pressure of predation birds have to undergo in natural conditions, it is not surprising if they usually show high levels of fear reactions. The risk of predation is reduced in domestic animals and completely disappears in domestic birds kept in intensive systems.

Expression of fear reactions in domestic animals has been reduced through selection (Faure et al., 1990; Mignon-Grasteau et al., 2005). However it is still sometimes too high for ease of care of such animals and can still be improved (Faure and Mills, 1998). Excessive levels of fear can lead to problems such as panic and hysteria (Mills and Faure, 1990) in laying hens and can also be responsible for welfare and production issues (Faure et al., 2003). Furthermore, freedom from fear is one of the five freedoms that should be guaranteed if good welfare conditions are to be achieved (FAWC, 1993).

When the selection reported here was undertaken some reports had already been published showing that selection for high or low expression of fear was possible in birds but the results were either short term (Gallup, 1974) or included selection for other motivational systems (Bourdens, 1971; Faure and Folmer, 1975; Faure, 1981; Faure et al., 1983; Faure et al., 2003).

Experiments have been undertaken in our laboratory to select for intensity of fear reaction as measured by duration of tonic immobility (TI). TI being chosen as a selection criterion because of its demonstrated relationship with fear (Faure et al., 1983; Jones, 1986), and the quail was chosen as a model because it is highly suitable for studies of genetics.

Quail have a limited space requirement (in contrast to requirements of the most recent EU regulations on welfare of experimental animals (EU, 2003)).

Food cost for quail are limited.

The quail is very resistant to all kinds of pathogen (in the poultry research station environment we have never had any health problems, without any vaccination or treatment other than an anticoccidian during the first 3 weeks of life).

The characteristics of the quail’s egg are very variable (size, shape, background colour, colour, size and shape of the dots) but each female lay very similar eggs throughout its life. It is thus possible to identify each female’s eggs in a two female group.

A female quail has a mean of 2.5 offspring per week, and the eggs laid over two weeks may be cumulated in the same batch. Furthermore, in contrast to mammals, it is very easy to have large batches born on the same day.

The quail is a good model for poultry, particularly as far as fear is concerned (Jones et al., 1982; Mills and Faure, 1986).

Selection for tonic immobility

All the characteristics of the animals and the breeding, testing and selection procedures used in our experiments are described in Mills and Faure, (1991). In brief, a three-way cross between heavy French commercial lines was used as the base population. The birds were reared in collective cages from hatching to 6 weeks of age and in pedigree cages (1 male, 2 females per cage) thereafter. The light regimen was 24 hours light during the first 3 weeks, to allow the hatchlings to find the Infra-Red brooder easily, 8L:16D from 3 to 6 weeks of age (to avoid early laying) and 16L:8D for adult animals. Food and water were provided ad-libitum throughout all their lives. From the first generation (F1) onwards, three lines were kept in parallel: a long tonic immobility line (LTI), a short tonic immobility line (STI) and an unselected control line (C). Each line was represented by about 200 hatchlings, of which 20 males and 40 females were used for reproduction. All the birds were pedigree hatched and wing-banded on hatching.

For the selection procedure, tonic immobility (TI) tests were performed in a V-shaped cradle when the birds were 9-10 days old. The maximum duration of TI was limited to 300 sec and up to 5 attempts at TI induction (number of inductions: NI) were performed if TI failed to occur (TI duration of less than 10 s). If a bird was not in TI after 5 attempts its NI score was 5 and TI duration 0. A TI duration of 300 s was recorded for birds which were still in TI after 300 s. All the chicks from the selected lines were measured, but only a sample of about 80 chicks was measured in the control line. In parallel with this selection for TI, other lines were also selected for social reinstatement behaviour (Mills and Faure, 1991) and the birds from the lines selected for TI were also measured for their social reinstatement behaviour.

TI duration was first regressed on the score for social reinstatement behaviour and individuals were selected on the basis of the resulting combined score to try to keep the score for social reinstatement constant over generations. An estimate of the breeding value of each individual was

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obtained by the combination of its individual phenotypic value and the mean of its siblings (Cochez and Pero, 1954; Falconer, 1961).

In the two selected lines, the selected birds were chosen on the basis of their estimated breeding value and care was taken to avoid excessive inbreeding (for each male no more than 2 sons and 4 daughters were chosen for the production of the following generation) up to the 16th generation. Thereafter selection was intra-family (1 son per male and one daughter per female). In the C line one son per male and one daughter per female were randomly chosen at each generation. Sibling mating was avoided at all times. After the 16th generation only one generation out of two was measured and thus selected. In the unselected generations of the selected lines, breeders were chosen randomly as in the C line.

Results of selection for tonic immobility

Mean TI duration increased from about 50 s in the base population to over 200 s after 20 generations of selection and then varied between 200 and 250 s in the LTI line. It decreased to about 10 s in the STI line and remained reasonably stable in the C line (Figure 1). However, these figures clearly result from ceiling and floor effects and TI duration was far higher in the LTI line if measured in unlimited time conditions. For example mean TI was about 450 s at the 13th generation when the maximum duration allowed was 1800 s (Launay, 1993; Launay et al., 1993). Similarly the mean NI necessary to induce TI was almost 1 in the LTI line while it was over 3 in the STI line, with a very high proportion of birds with 5 unsuccessful inductions. It should be emphasized that the procedure used in our experiments for TI testing has been that followed by most authors working on the subject since Gallup (1979). The fact that induction is repeated if unsuccessful, more or less implies that the responsibility for unsuccessful induction is that of the experimenter however successful selection of this characteristic shows that it is at least partly inherent to the bird genotype.

TI duration is age dependent but the differences between lines persist whatever the age of the quail between 1 and 10 weeks of age (Launay, 1993), i.e. even in adult quail.

Research into genetic mechanisms involved

Realised heritabilities obtained over the first 8 generations ranged between 0.2 and 0.3 (Mills and Faure, 1991). QTL (Quantitative Trait Loci) research was undertaken on a F2 cross and 1048 F2 quails measured for their TI characteristics (NI and TI duration), open-field behaviour and stress susceptibility (Beaumont et al., 2005). Selective genotyping was achieved on 326 birds issuing from the 6 males most likely to be heterozygous according to a segregation analysis of the whole data sets (i.e. the 9 male and 18 female F1 and their 1048 F2 offspring). AFLPTM markers were derived as described by Roussot (2003) and used. The analysis was based on the method developed for this type of dominant marker (Perez-Enciso and Roussot, 2002) using all available data to differentiate between animals that were homozygous or heterozygous for the marker.

Several QTL were identified; those related to Tonic immobility are summarised in Table 1. They represent 5 linkage groups and the first two are located on the first linkage group at a distance of 55 cM. Other QTLs related to open-field behaviour were also found (Beaumont et al., 2005). Except for the QTL found on linkage group number 10, all the others were close or possibly identical to QTLs controlling open-field behaviour. This result strengthens the probability that genes coding for fear susceptibility are closely associated with these QTL and this result is consistent with the differences observed between the two lines (Faure et al., 2003) and with the correlations (although low) observed between these traits (Mignon-Grasteau et al., 2003). Further investigations are needed to establish whether some of these QTLs might be related to those controlling stress reactions (Schütz et al., 2004) or pecking behaviour (Buitenhuis et al., 2003) in chickens.

Consequences of selection for tonic immobility

Behaviour

In a complex experiment involving LTI and STI lines and also handling and enrichment treatments, and testing the birds in open-field, emergence and TI tests, all the parameters measured were significantly affected by the line (except the number of jumps in the open-field test). In every case LTI birds showed longer latencies, uttered fewer cries and showed lower activity levels than STI quails (Jones et al., 1991). In a test where chicks were presented with a conspicuous bead, LTI chicks showed higher inhibition of head pecking than STI chicks (Richard et al., 2000). All these characteristics show that LTI quail express higher fear reactions than STI birds. Similarly, Richard-Yris et al. (2004) and Bertin (2005) showed higher fear reactions to the approach of man in LTI than in STI quail.

In contrast to chickens, quails are easy to capture in a pen because they tend to come close to man (Bessei et al., 1983). A capture experiment was conducted in a pen where STI and LTI quail were kept together. In such condi-

![Figure 1. Results of 40 generations of divergent selection for duration of tonic immobility (STI: Short duration of tonic immobility; C: Unselected control line; LTI: Long duration of tonic immobility)](image)

Ergebnisse der divergierenden Selektion auf die Dauer der tonischen Immobilität über 40 Generationen (STI: kurze Dauer der tonischen Immobilität; C: unselektierte Kontrolle; LTI: lange Dauer der tonischen Immobilität)
tions it is assumed that the more familiar birds, i.e. those closest to the experimenter, will be captured first. In these conditions, STI quail were captured first (MILLS and FAURE, 2000).

In an experiment where groups of young quails where faced with either normal or coloured food in the presence or absence of companions used to eating coloured food, LTI birds showed a longer latency to eat in the absence of companions whereas STI quails showed a short latency to eat in both conditions. Food neophobia was expressed only by isolated LTI quails and the presence of experienced companions reduced the latency to eat in LTI quail to the level observed in the other three situations (LAUNAY, 1993).

In most of these experiments quails were either placed in a novel environment for the experimental procedure, and were then manipulated shortly before the test, or were tested for their fear of humans. In one experiment where STI and LTI quail were tested for their fear of an object that was introduced into the rearing cage by an experimenter out of bird sight, no difference in fear reaction was observed between the lines (WACRENIER, 2005). Whether this was due to the test being performed in a familiar environment or to the absence of human involvement was not clear.

The two selected lines also differ in the speed at which adult females adopt young quail chicks, with STI quail adopting more quickly than LTI quail (RICHARD-YRIS, 1994). Quail chicks from an unselected line adopted by LTI mothers also consistently show greater fear reactions than those adopted by STI mothers (RICHARD-YRIS et al., 2004; BERTIN, 2005).

Surprisingly LTI males have been reported consistently to show shorter latency to display and copulate and more active sexual behaviour than STI birds (PORTEOUS, 1992; BURNS et al., 1998). This is not consistent with previous results and a genetic drift for sexual behaviour is the only explanation we can propose.

LTI females are more sexually receptive than STI females when tested in their familiar environment. The reverse is true when tests are performed in an unfamiliar environment. In the latter case STI females show strong territorial behaviour that inhibits male sexual behaviour (PORTEOUS, 1992).

One surprising phenomenon is the fact that quails of both lines show a sexual preference for individuals of their own line, and this is the case even when birds are reared from hatching with mixed lines (PORTEOUS, 1992).

### Physiology

**Central Nervous System.** At the level of the brain, two main approaches have been used to compare STI and LTI quail, one being pharmacological and the other neuroanatomical.

Various neurotransmitter systems have been investigated in the pharmacological approach, and adrenergic, serotonergic, opioid and benzodiazepine binding parameters in the forebrain were compared between STI and LTI quail (HOGG et al., 1994). No difference was found in α2, 5-HT1A, 5-HT3, µ- and κ-opioid receptor binding between the two lines. The LTI line had fewer δ-opioid receptors than the STI line, but the two lines did not differ with respect to the affinity of these receptors. Finally, the two lines did not differ in number of benzodiazepine receptors but LTI quail exhibited lower affinity than STI quail for benzodiazepine ligands at the diazepam-sensitive binding site. Subsequent analysis revealed that STI and LTI quail differed greatly in the neurochemical properties of several components of benzodiazepine binding, notably at the level of the GABA<sub>δ</sub>-benzodiazepine complex (HOGG et al., 1996). Thus, the selective breeding of the two lines has resulted in differences in benzodiazepine and δ-opioid binding, which could contribute to the behavioural differences in fear responses exhibited by the two lines.

The neuroanatomical approach has focused on a telencephalic region known to be involved in the control of fear behaviour in birds, formerly called the archistriatum and recently subdivided into arcopallium and posterior pallial amygdala (REINER et al., 2004). Bilateral lesions of the archistriatum significantly reduced fear behaviour scores in LTI quail chicks, as measured in the emergence and tonic immobility tests, but they did not appear to have any significant effect on fear behaviour reactions in STI quail chicks (DAVIES et al., 1997). When tested as adults, lesioned LTI quail continued to exhibit reduced fear behaviour: they showed significantly less avoidance of a novel object and
Autonomic Nervous System. Investigations have also been carried out on the autonomic nervous system of the selected lines since autonomic activity is highly involved in stress responses (Gaudinier et al., 2002, 2005). The autonomic nervous system is constituted of two sub-systems: the sympathetic and the parasympathetic nervous systems. Both sub-systems regulate several organs, particularly the heart.

Heart activity varies extremely quickly and analysis of this variability has allowed evaluation of the respective influences of the sympathetic and parasympathetic nervous systems on the quail heart. For these investigations, quail were surgically fitted with telemetric implants, which recorded electrocardiograms without restraining the birds.

The first study investigated whether genetic selection on duration of tonic immobility was related to changes in the neural control of the heart. Autonomic control of the heart was assessed by pharmacological blocking followed by heart rate variability analyses. Heart rate before injection did not differ between the LTI, STI and C lines. Analysis of heart rate variability demonstrated that quail from the three lines had the same intrinsic tone but different autonomic activity. Parasympathetic activity was predominant in control and in STI quail whereas there was a balance between sympathetic and parasympathetic activity in LTI quail (Figure 2). Moreover, STI quail differed from control quail in parasympathetic activity whereas LTI quail differed from control quail in sympathetic activity (Figure 2).

Selection on duration of tonic immobility thus appears to be associated with changes in the sympathetic-vagal control of the heart, which may influence behavioural responses to stressful situations.

In the second study, an acoustic stimulus was used to study autonomic responses to a stressful situation. Two experiments were performed. The first experiment was designed to look for correlations between duration of TI and heart rate variability in response to the acoustic stimulus. The second experiment compared motor and autonomic responses of STI and LTI quail to the acoustic stimulus. The first experiment showed that the acoustic stimulation induced motor inhibition and cardiac activation. Correlations were found between duration of tonic immobility and both autonomic activity before stimulation and sympatho-vagal balance after stimulation. In the second experiment, LTI quail showed strong sympathetic activation whereas STI quail showed simultaneous parasympathetic and sympathetic activation. The activation of the parasympathetic system induced by the noise in STI quail may be explained by the predominance of fibers in this line.

In conclusion, both the basal autonomic activity and the autonomic responses differed according to the emotional reactivity of the quail and changes in autonomic activity appear to be related to the genetic selection process.

Hypothalamo-pituitary-adrenal (HPA) axis. Activation of the HPA axis is part of the major physiological mechanisms that contribute to the regulation of homeostasis, especially in the context of stressful events, and for this reason its functioning has been thoroughly investigated. No line difference was observed in any of the experiments conducted in terms of basal plasma levels of corticosterone (CORT). After a mild stressor such as the introduction of a novel object in the cage, a slight increase in CORT level was observed only in LTI quail (Launay et al., 1993) or in both lines (Hazard, 2005; Wacrenier-Cere, 2005). In an experiment where LTI and STI females were repeatedly socially isolated in an unfamiliar environment, Porteous (1992) observed a weight loss in both lines but the loss, probably resulting from the stress induced by the repeated handling and isolation, was greater in STI quail. However, a single application of this treatment only induced a slight increase in CORT level, of similar amplitude for both genotypes (Hazard, 2005).

Restraint of quail in a crush cage (Satterlee and Johnson, 1988) was found always to induce a significant increase in CORT level in both lines. However, the increase was much greater in STI than in LTI quail (Hazard et al., 2005a and b) and the difference lasted for at least two hours in some cases (Hazard, 2005). There was, however, no difference between the two lines for the sensitivity of the adrenal gland to 1-24 ACTH and the dose-response curves of CORT to 1-24 ACTH injections were similar for the two lines at 6 weeks of age (Hazard, 2005; Hazard et al., 2005a and b). Furthermore, HPA axis responsiveness to restraint and adrenal responsiveness to 1-24 ACTH injection remained stable in STI quail, whatever the age between 4 and 12 wks. On the other hand, in LTI quail responses to restraint decreased and adrenal responsiveness increased with age (Hazard et al., 2005b). In the crush cage, the two lines showed very different behaviour reactions, STI birds initially showing a greater number of escape movements, with progressive decline, whereas LTI quail showed very few escape movements from the beginning of the test (Hazard, 2005). The difference in CORT level thus does not only reflect this difference in activity.

The divergent selection program for TI conducted on quail has resulted in differences in CORT response to restraint as well as in changes in HPA responsiveness with age in STI quail. These differences probably resulted from upstream structures of the HPA axis and differences in development rate of the adrenal gland, respectively.

In another set of experiments, various aspects of post-mortem muscle physiology were observed after 3 types of stressor, i.e. restraint in a crush cage, fasting and
transportation. Despite the fact that LTI quail showed the lowest increase in plasma levels of CORT they also showed higher pH values in the pectoralis major muscle 24 hours post-mortem in all three conditions and higher drip loss from the same muscle after stress in the crush cage only. These parameters are indicative of poor meat quality (dark, firm and dry meat) in LTI quail after stress (Remignon et al., 1997).

**Productivity.** STI quail are heavier, have lower age at first egg and lay more but smaller eggs than LTI quail (Minvielle et al., 2002).

**Conclusions**

Divergent selection for duration of TI has resulted in divergence for many behavioural and physiological characteristics, particularly those related to expression of fear and stress reactions. It has also resulted in differences in brain structure and metabolism. These results show that it is possible to select for reduced fear and thus have animals that are easier to care for, with improved welfare and at the same time some beneficial consequences on production parameters. Moreover, STI and LTI lines constitute a unique tool for investigating the mechanisms underlying fear behaviour in birds.

**Summary**

Selection for the duration of tonic immobility (TI) has now been performed in quail over 40 generations. After 20 generations of selection the mean duration of TI for the long TI line (LTI) was of over 200 s whereas for the short TI line (STI) it was about 10 s. Heritability values were in the range 0.2 to 0.3. QTL research identified 5 loci associated with duration of TI of which four were either identical or close to loci associated with open-field behaviour.

LTI quail consistently show stronger fear reactions than STI quail in many situations involving reactions to new environments, new objects or the proximity of man. The lines also differ at brain level, with functional differences in benzodiazepine and δ-opioid binding and also anatomical differences, particularly of the archistriatum. At rest STI quail show a predominance of the parasympathetic over the sympathetic system and a higher level of activation of the parasympathetic system after a startling stimulus (noise). The two lines do not differ in the basal levels of corticosterone but LTI quail show higher corticosterone levels after restraint in a crush cage. This difference cannot be completely explained by the difference in behaviour between the two lines in this situation. The reactivity of the adrenal gland to ACTH injection is similar in the two lines and the differences observed probably occur at hypothalamic and/or pituitary levels or higher.

These selection experiments have provided a very powerful tool to study the consequences of different fear levels on various aspects of the behaviour and physiology of the birds. They also show that selection for fear or stress susceptibility is possible in commercial lines and that there is the potential to obtain better animals in terms of ease of care and productivity with simultaneous improvement in welfare.

**Key words**

Japanese quail, genetics, selection, behaviour, fear

**Zusammenfassung**

**Auswirkungen der Selektion auf Furcht bei der Japanischen Wachtel**


**Stichworte**

Japanische Wachtel, Gentik, Selektion, Verhalten, Furcht

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