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**Original article**

# Quantitative genetics of growth traits in the edible snail, *Helix aspersa* Müller

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**Summary** – Genetic parameters of adult weight, age at maturity (adult age), weight after hibernation and relative loss of weight during hibernation were estimated in a population of edible snails (*Helix aspersa* Müller). Eight thousand four hundred and eighthy three animals were sampled from 143 pairs for adult weight, 4 333 from 87 pairs for adult age and 2 256 from 123 pairs for traits after hibernation. An animal model taking into account all the relationships was used to estimate genetic parameters. Estimates were also computed from the covariances between full-sibs and parent offspring regressions to assess possible non-additive genetic effects. Heritabilities were high except for relative loss of weight during hibernation. Estimates from the animal model were 0.48 ± 0.04 for adult weight, 0.40 ± 0.05 for adult age, 0.40 ± 0.05 for weight after hibernation and 0.12 ± 0.03 for relative loss of weight during hibernation. Adult weight and adult age were neither phenotypically nor genetically correlated (0.05 and 0.003 ± 0.07, respectively). A substantial maternal effect, especially on adult weight was found.

**growth / heritability / genetic correlation / *Helix aspersa***

**Résumé – Génétique quantitative des caractères de croissance chez l'escargot comestible, *Helix aspersa* Müller.** *Les paramètres génétiques de plusieurs caractères de croissance ont été estimés dans une population d'escargots Petit-Gris (*Helix aspersa *Müller). Il s'agit du poids adulte, de l'âge à maturité (âge adulte), du poids après hibernation et de la perte relative de poids lors de l'hibernation. Le nombre d'observations collectées se répartit ainsi : 8 483 animaux issus de 143 couples pour le poids adulte, 4 333 issus de 87 couples pour l'âge adulte et 2 256 issus de 123 couples pour les caractères mesurés après hibernation. Afin de tenir compte de toutes les relations de parenté, nous avons utilisé un modèle animal pour estimer les paramètres génétiques. Ils ont également été estimés à*

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*partir des covariances entre plein-frères et de la régression parents-descendants. Cela nous a permis de discuter des effets génétiques non additifs. Tous les caractères sauf la perte de poids relative lors de l'hibernation révèlent des héritabilités élevées. Les estimations issues du modèle animal sont de 0,48 ± 0,04 pour le poids adulte, 0,40 ± 0,05 pour l'âge adulte, 0,40 ± 0,05 pour le poids après hibernation et 0,12 ± 0,03 pour la perte relative de poids lors de l'hibernation. Il n'y a pas de corrélation (ni phénotypique, ni génétique) significative entre le poids et l'âge adultes (0,05 et 0,003 ± 0,07, respectivement). Nous avons également mis en évidence un effet maternel important, en particulier sur le poids adulte.*

**croissance / héritabilité / corrélation génétique / *Helix aspersa***

## INTRODUCTION

Each year, about 25 000 tons of snails (*Achatina* and *Helix* genus) are imported into France. French production has quickly developed since 1980: from 10 tons in 1985 to about 400 tons in 1994. The species reared is *H aspersa*. Rearing methods have been improved, and now efficient selection programs are needed to increase the profitability of snail farming. An accurate estimates for genetic parameters would help to set up such selection programs.

However, very little research has dealt with quantitative genetics of land snails (for a review, see Dupont-Nivet et al, 1997). Only estimations of shell size were reported and mostly concerned species other than *H aspersa*. Moreover, most of these estimates were based on limited data and biased by some environmental effects.

More reliable estimates for *H aspersa* weight and shell size heritabilities were given by Dupont-Nivet et al (1997). However, in this paper, attention was mainly focused on genetic parameters of other economically related traits, such as adult age. The aim was to obtain enough data to estimate genetic parameters more accurately.

The present study was carried out to obtain accurate estimates for the main growth traits, ie, adult weight, age at maturity (adult age), weight after hibernation and relative loss of weight during hibernation. Environmental factors were studied and genetic parameters were estimated using three different methods: full-sib covariances, mid-parent/offspring regression and animal model. The last method delivered the most accurate estimates since all relationships between relatives are taken into account, provided that all genetic effects are additive ones. The two other methods allowed a check for the importance of non-additive genetic effects.

### *Specificities of snail biology and experimental breeding*

#### Growth of snail

Most retailed animals are adult animals. A snail is an adult when the shell peristome (shell edge) is reflected, ie, when the shell growth is completed. In conventional snail farms, growth until adult-age stage takes 4–6 months. However, lower population density leads to lower mean adult age (see below). Two measurements of adult size are available: adult weight and shell diameter. An adult *H aspersa* weighs between 6 and 15 g. Snail weight can change according to its water content (Le

Guhennec, 1985; Klein-Rollais, 1990) while shell diameter is less variable. Indeed, Albuquerque de Matos (1989) showed that successive measures of shell diameter do not differ by more than 0.5 mm (ie, about 1% of the average size). Yet, from a breeding standpoint, weight is much more important than diameter. As shown in Dupont-Nivet et al (1997), shell diameter and adult weight are highly correlated (phenotypically and genetically), with similar fixed effects and heritabilities. Thus, weight as well as shell diameter may be chosen to characterize adult size.

Compared to conventional domestic livestock, mortality during growth is high and extremely variable (5–50%). Snail pathology is poorly known. As a result, detection of diseases, investigation of causes of death or prevention against pathologies remain difficult except for basic care such as disinfection.

## Reproduction of snail

No external sign of sexual maturity is known. Therefore, it was assumed that snails with reflected peristome were sexually mature and they were used for reproduction.

*H aspersa* is a protandrous hermaphrodite. Mating occurs between two male snails which fertilize one another. Most often, mating lasts more than 10 h. Then, both partners turn into females and lay eggs. This takes a few days to several weeks and hatching takes 10–25 days. In laboratory conditions, *H aspersa* mates twice or three times on average, and lays 1.5 times, ie 120–130 eggs (Madec and Daguzan, 1993).

This is the usual reproduction cycle. However, some snails mate several times before laying, while others never mate. If a snail has not laid five weeks after mating, it is considered to be a non-layer. Laying pairs, where only one snail lays are called 'unilateral' pairs, and are called 'bilateral' pairs if both snails lay.

Hermaphroditism makes it possible to estimate a reciprocal effect. In bilateral pairs, offspring of both partners are full-sibs but maternal effects are different, allowing us to estimate a reciprocal effect by comparing clutches within each pair.

Mating takes place in reproduction boxes such as those described in Bonnet et al (1990). Snails can store sperm from different partners and may lay eggs from several matings in a same brood (Murray, 1964). To avoid multiple matings and to warrant the reliability of pedigrees, snails are isolated into laying boxes as soon as they have been seen copulating. When mating is over, snails are isolated from one another and given an egg-laying jar (9 cm diameter garden pot, filled with soil).

## Experimental conditions

During reproduction and growth, animals were housed in rooms, located in two adjacent buildings, where the following characteristics were kept constant:

light/darkness cycle: 16L:8D;

temperature: 20 °C in the day and 17 °C in the night with correspondingly 70% and 90% relative humidity.

Animals were fed ad libitum with a commercial compound feed (crude protein 15%, crude fat 2%, cellulose 3%, ash 37%). Breeding boxes were cleaned and food renewed once a week.

### Hibernation

In the laboratory, we cannot as yet synchronize snail reproduction. In the best cases, time between the first and the last mating was about 2 months. The interval between mating and laying ranged from several days to more than 4 weeks. This led to a very important heterogeneity of snail birth dates. Growth duration was also highly variable and adult snails were obtained at very different dates. For practical reasons (unwanted matings and mortality), they could not be kept in growth boxes.

Hibernation allowed us to store snails between the end of growth and the beginning of reproduction. Moreover, Aupinel (1984) has shown that a hibernation of at least 3 months enhances reproduction performances.

As soon as they reached adult size, animals were put into a cold chamber for hibernation (temperature: 5 ± 1 °C, relative humidity: 80%, light/darkness cycle: 6L:18D) before reproduction.

## MATERIALS AND METHODS

As a large number of animals was required to achieve precise estimates of genetic parameters and since facilities were limited, data from animals of three successive generations (called G1, G2 and G3) were used in this work.

### *Snails*

G0 snails were collected in the wild. The sampling design was a compromise between the following two requirements.

Several colonies had to be sampled in order to obtain unrelated snails and to avoid a founder effect. Indeed, most of the snail populations are highly polymorphic, but isolated colonies with high inbreeding and little polymorphism may be found (Madec, 1991, Guiller et al, 1994).

Sampling too distant populations should be avoided to minimize linkage disequilibrium and heterosis under crossbreeding. However, enzymatic studies (Guiller et al, 1994) showed that snail populations within the same region are not very distant genetically.

Therefore, the parents of G1 were 500 wild animals (G0), sampled in 1992 in 20 different locations of Poitou-Charentes (France), distant by at least 1 km. There was no voluntary selection during this experiment.

### *Reproduction*

In G0, snails were divided into five reproduction boxes of 100 snails, so as to minimize the number of snails from the same colony in the same box and therefore matings between possibly related snails.

Offspring (full-sibs) of a pair constituted a family. In G1 and G2, snails used for reproduction were randomly sampled from all families to preserve genetic variability. Animals were divided into 14 (G1) or 25 (G2) reproduction boxes with 56 (G1) or 59 (G2) animals per box. A given box contained only one snail from each family to avoid full-sib matings. In addition, some boxes (four in G1 and ten in G2)

hosted snails from only three (G1) or two (G2) families, to obtain full-sib matings and to study inbreeding effects on adult weight and age. However, offspring from those matings were not used for reproduction. Frequencies of the different types of matings and egg-layings obtained in each generation are shown in table I.

**Table I.** Number of different kinds of matings observed in each generation (including matings for which offspring were not reared).

| | *G0* | *G1* | *G2* |
|---|---|---|---|
| Breeding animals | 500 | 784 | 1 475 |
| Breeding boxes | 5 | 14 | 25 |
| Matings[a] | 133 | 334 | 576 |
| Full-sib matings | – | 54 | 138 |
| Egg-layings[b] | 129 | 466 | 750 |
| Unilateral egg-layings | 21 | 136 | 196 |
| Bilateral egg-layings | 108 | 330 | 554 |
| Full-sib egg-layings | – | 73 | 191 |

[a] One mating = two mated animals; [b] one egg-laying = one egg-laying laid by one animal.

### *Growth*

As room was lacking to raise all clutches, some were randomly discarded. From each clutch, only 75 (G1 and G2) or 50 (G3) animals were reared. They were randomly picked out from the whole clutch. Since young snails could not be shell-tagged, broods could not be mixed, and it was necessary to estimate a 'box' effect. For that purpose, G1 and G2 snails of each clutch were divided into three groups, each of them being reared in a different box. After having discarded one group at random from G1 and G2 data, we again estimated heritabilities and fixed effects with the 'pair' model (see below). As results were not significantly different, we used only two groups for G3.

Batches of 25 newly hatched snails were grown to adult stage in wooden boxes measuring 25 × 12 × 40 cm (Bonnet et al, 1990). Snails that reached adult stage very late (after 5 months of growth) were eliminated from our experiment.

### *Hibernation*

Animals were kept in hibernation from the time they reached adult age until the reproduction stage. Thus, the duration of hibernation was determined by both biological variables (birth date and growth length) and by management considerations (the choice of a date of reproduction). Therefore, the date when snails started hibernation was highly variable but the end of hibernation was the same for all snails used for reproduction.

### *Traits measured and analyzed*

For each generation the corresponding sample sizes with respect to each trait are specified in table II. Once a week, adults were removed from growth boxes. Adult age (G2 and G3) and adult weight (G1 to G3) were recorded. In G2 and G3, to standardize measurement of weight, snails were weighed just after reaching adult age and after a 3-day fasting in wooden boxes under dry atmosphere (Dupont-Nivet et al, 1997). Animals withdrawn from hibernation for reproduction were weighed so that weight after hibernation (WAH) and relative loss of weight during hibernation (RLWDH) could be analyzed. All weights were measured to the nearest 0.01 g with a METTLER balance. We also counted the number of eggs per clutch and weighed each clutch with a METTLER balance (to the nearest 0.01 g) in order to calculate the egg mean weight. Egg mean weight was used to study maternal effects (see below).

**Table II.** Characteristics of the data sets used for analyses.

| *Traits* | | *G1* | *G2* | *G3* | *Total* |
|---|---|---|---|---|---|
| Adult age | animals/families[a] | – | 3556/68 | 777/22 | 4333/90 |
| | animals from bilateral layers/ bilateral families | – | 2537/47 | 566/12 | 3103/59 |
| | inbred animals/ inbred families | – | 287/9 | 142/4 | 429/13 |
| Adult weight | animals/families | 4150/56 | 3556/68 | 777/22 | 8483/146 |
| | animals from bilateral layers/ bilateral families | 3672/47 | 2537/47 | 566/12 | 6775/106 |
| | inbred animals/ inbred families | 0/0 | 287/9 | 142/4 | 429/13 |
| Weight after hibernation | animals/families | 784/56 | 1475/67 | – | 2259/123 |
| | animals from bilateral layers/ bilateral families | 658/47 | 990/46 | – | 1648/93 |
| Relative loss of weight during hibernation | animals/families | 784/56 | 1475/67 | – | 2259/123 |
| | animals from bilateral layers/ bilateral families | 658/47 | 990/46 | – | 1648/93 |

[a] A family is composed of all full-sibs born from the same pair of snails.

### *Statistical analyses*

All calculations except animal model analysed were made with the SAS® Institute computer package (1987). Performances of G0 animals were only used for the regression analysis.

Normality of variables was studied by computing the Kolmogorov test and by considering skewness and kurtosis. All but two variables (adult age and relative loss of weight during hibernation) seemed very close to normality even if the test rejected the hypothesis of normality (table III). For these two variables, several transformations were tested to approach normality. A reciprocal transformation for adult age and a square root transformation for relative loss of weight during hibernation gave the best adjustments. Differences between the estimates of genetic parameters for initial and transformed variables were lower than the standard errors. It was decided that such differences were negligible and only results from initial variables have been shown.

Phenotypic correlations were Pearson's correlations. Both traits measured after hibernation were significantly correlated with the hibernation duration. Moreover, adult age partly determined the hibernation length (see *Material and methods – Hibernation*). To eliminate this automatic correlation between adult age and WAH and RLWDH, WAH and RLWDH were linearly corrected by the hibernation length.

The data may be influenced by the following effects: year effect, room effect, unilateral/bilateral laying-pair effect, inbreeding effect, box effect, reciprocal effect and relationships between the animals. A 'pair' model was used to study the significance of effects and to estimate heritabilities.

### 'Pair' model

The following model was used for traits measured before hibernation:

$$Y_{ijklmnop} = \mu + A_i + UB_j + B_k + I_l + G_{ijklm} + R_{ijklmn} + M_{ijklmno} + E_{ijklmnop}$$

where $Y_{ijklmnop}$ was the trait measured of the $p$th animal in the $ijklmno$th class, $\mu$ the overall mean, $A_i$ the fixed effect of the $i$th year, $UB_j$ the fixed effect of unilateral/bilateral laying-pair, $B_k$ the fixed effect of the $k$th room, $I_l$ the fixed effect of inbreeding (with two classes: progeny from full-sib mating or not), $G_{ijklm}$ the random effect of the $m$th pair, $R_{ijklmn}$ the random reciprocal effect, $M_{ijklmno}$ the random effect of the $o$th box of growth and $E_{ijklmnop}$ the residual error. For traits measured after hibernation, the following model was used:

$$Y_{ijklm} = \mu + A_i + UB_j + G_{ijk} + R_{ijkl} + E_{ijklm}$$

where $Y_{ijklm}$ was the trait measured of the $m$th animal in the $ijkl$th class, $\mu$ the overall mean, $A_i$ the fixed effect of the $i$th year, $UB_j$ the fixed effect of unilateral/bilateral laying-pair, $G_{ijk}$ the random effect of the $k$th pair, $R_{ijkl}$ the random reciprocal effect and $E_{ijklm}$ the residual error. The growth box effect was not introduced because it was confounded with the residual effects, and no inbred animals were used for breeding so that no inbreeding effect was considered in this model.

These models allowed one to investigate whether fixed effects were significant and to estimate variance components of random effects using the SAS mixed procedure with the REML method. The ratio $\sigma_f/\sigma_e$ was calculated where $\sigma_f$ is the standard deviation of the random factor and $\sigma_e$ is the residual standard deviation. This ratio asseses the amount of each random effect in the variability of the traits studied. The models analyzed data from full-sib animals. The pair effect represents the full-sib family effect. Therefore, if genetic effects are additive:

$$\sigma_g^2 = 1/2\sigma_a^2$$

where $\sigma_g^2$ is the variance of the pair effect and $\sigma_a^2$ is the additive genetic variance. Thus, heritabilities ($h^2$) were estimated by:

$$h^2 = \frac{2\sigma_g^2}{\sigma_y^2}$$

where $\sigma_y^2$ is the phenotypic variance.

Standard errors (SE) of the heritabilities were estimated as indicated by Becker (1984).

'Pair' models use only full-sib relationship. An animal model was used to take all relationships into account.

## Animal model

A multiple trait animal model was used to compute REML estimates with VCE version 3.2 (Groeneveld, 1996):

$$\mathbf{Y} = \mathbf{X\beta} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{r} + \mathbf{Z}_3\mathbf{m} + \mathbf{e}$$

where $\mathbf{Y}$ was the vector of observations, $\boldsymbol{\beta}$ the vector of fixed effects (except inbreeding effect), which were shown to be significant by the 'pair' model, $\mathbf{a}$ the vector of random animal effects $(\mathbf{0}, \mathbf{A}\sigma_a^2)$, $\mathbf{r}$ the vector of random reciprocal effects $(\mathbf{0}, \mathbf{I}\sigma_r^2)$, $\mathbf{m}$ the vector of random box effects $(\mathbf{0}, \mathbf{I}\sigma_m^2)$ and $\mathbf{e}$ the vector of random residual effects $(\mathbf{0}, \mathbf{I}\sigma_e^2)$. $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$ and $\mathbf{Z}_3$ were the incidence matrices relating observations to the appropriate effects, $\mathbf{A}$ was the numerator relationship matrix, $\mathbf{I}$ was the identity matrix.

All covariances between random effects were set to zero. This model assumed that genetic effects were only additive. To test the existence of non-additive genetic effects, heritabilities were also estimated by regression.

## Regression

Heritabilities were estimated through a regression of mean performance of offspring on parental midvalues. Data were corrected for the non-genetic effects found to be significant in the pair model. Families with less than five offspring were discarded. The regression was computed with equal weights for all families. According to Falconer (1989), this estimate is not biased by the dominance and maternal effects, but remains slightly biased by epistatic effects. However, under a purely additive model, it is less precise than estimations from the animal model.

## RESULTS

Basic statistics are reported in tables III and IV. All mean measurements except mean adult age were in agreement with those of Madec and Daguzan (1993) and Bonnet et al (1990). It is worth noting that all traits have large coefficients of variation (from 20.4% for adult weight to 33.2% for RLWDH).

**Table III.** Basic statistics (1): before transformation; (2): after transformation (reciprocal for adult age and square root for relative loss of weight during hibernation).

| | *Mean* | *Standard deviation* | *Coefficient of variation* | *Skewness* (1) | (2) | *Kurtosis* (1) | (2) | *Kolmogorov test* (1) | (2) |
|---|---|---|---|---|---|---|---|---|---|
| Adult age (week) | 12.2 | 2.7 | 21.5 | 1.16 | −0.07 | 1.25 | −0.18 | 0.18 | 0.13 |
| Adult weight (g) | 9.65 | 2.0 | 20.4 | 0.51 | – | 0.65 | – | 0.04 | – |
| Weight after hibernation (g) | 7.40 | 1.6 | 21.6 | 0.50 | – | 0.72 | – | 0.04 | – |
| Relative loss of weight during hibernation (%) | 21.4 | 7.1 | 33.2 | 0.44 | −0.06 | 4.68 | 0.83 | 0.04 | 0.01 |

The correlation between adult age and adult weight was very low (0.05, $P < 0.01$). Most of the phenotypic correlations were consistent with the breeding cycle, such as the correlations between WAH and RLWDH, WAH and hibernation length, RLWDH and hibernation length. The correlation between adult age and hibernation length was highly negative (−0.7). The correlation sign was consistent with the fact that snails were put into hibernation after they became adult and snails allowed to breed were all removed from hibernation at the same time. Thus, the younger the snail when it became an adult the longer it was kept in hibernation. However, the correlation did not reach −1 since snails from different families were born at very different dates (more than 3 months between the first and the last new born of the same generation). In addition, adult age ranged from 6 to 20 weeks, even within family.

Significance levels of fixed effects and ratios $\sigma_f/\sigma_e$ of random effects are reported in tables V and VI, respectively. Year effect was very important for adult weight but with no clear trend over generations (10.44 g in G0; 9.35 g in G1 and 10.18 g in G3). Year effect was not significant for adult age. The unilateral/bilateral laying-pair effect was significant only for the two measurements of weight: offspring from unilateral pairs were bigger (+0.42 g for adult weight and +0.94 g for WAH). Offspring from sib-matings were not significantly less heavy than offspring from

**Table IV.** Phenotypic correlations.

| | *Adult weight* | *Weight after hibernation* | *Relative loss of weight during hibernation* | *Hibernation length* | *Weight after hibernation corrected for the hibernation length* | *Relative loss of weight during hibernation corrected for the hibernation length* |
|---|---|---|---|---|---|---|
| Adult age | 0.05<br>*<br>(4 333) | 0.28<br>***<br>(1 475) | −0.36<br>***<br>(1 475) | −0.70<br>***<br>(1 475) | 0.14<br>***<br>(1 475) | −0.17<br>***<br>(1 475) |
| Adult weight | | 0.90<br>***<br>(2 256) | 0.13<br>***<br>(2 256) | | | |
| Weight after hibernation | | | −0.30<br>***<br>(2 256) | −0.20<br>***<br>(2 256) | | |
| Relative loss of weight during hibernation | | | | 0.28<br>***<br>(2 256) | | |

Levels of significance: *, $P < 0.05$; ***, $P < 0.001$; sample sizes between brackets.

unrelated parents but became adult significantly later (12.3 against 11.4 weeks). A significant reciprocal effect was found for all traits, although less important for adult age and RLWDH. Box effect on adult weight was lower than the reciprocal and genetic effects, while for adult age the box effect and the reciprocal effect were almost equally important.

Estimates of heritabilities and genetic correlations are presented in table VII. Heritability estimates were high except for RLWDH. The estimates from the animal model were 0.40 ± 0.05 for adult age, 0.48 ± 0.04 for adult weight, 0.40 ± 0.05 for weight after hibernation and 0.12 ± 0.03 for relative loss of weight during hibernation. For adult age, estimates by different methods were similar (from 0.36 to 0.40). For the other traits, the estimates were highly different according to models. The animal model estimates were intermediate, whereas they were highest from the pair model estimates and regression yielded lowest values. For example, for adult weight, heritability estimates were 0.60 ± 0.07 from the pair model and 0.40 ± 0.08 from the regression model.

The genetic correlation between adult age and adult weight was not significantly different from zero (0.003 ± 0.07). The genetic correlation between WAH and RLWDH was surprisingly positive (0.50 ± 0.16) as opposed to phenotypic correlation (−0.30, $P < 0.001$). The genetic correlations between adult weight, WAH and RLWDH were consistent with the corresponding phenotypic correlations. However, the genetic correlation between adult weight and RLWDH (0.59 ± 0.10) was higher than the phenotypic one (0.13, $P < 0.001$).

**Table V.** F-values and probability of larger values for fixed effects.

| *Traits* | *Level of effects* | *Least square means* | *Pr(F)* |
|---|---|---|---|
| Adult age (weeks) | year: 2 | 12.18 | |
| | 3 | 11.56 | 0.09 |
| | building: 1 | 12.71 | |
| | 2 | 11.03 | 0.0013 |
| | laying-pair: unilateral | 11.97 | 0.52 |
| | bilateral | 11.77 | |
| | inbreeding: yes (full-sibs) | 12.32 | 0.04 |
| | no | 11.43 | |
| Adult weight (g) | year: 1 | 10.44 | |
| | 2 | 9.35 | |
| | 3 | 10.18 | 0.0001 |
| | building: 1 | 9.65 | |
| | 2 | 10.32 | 0.15 |
| | laying-pair: unilateral | 10.20 | |
| | bilateral | 9.78 | 0.06 |
| | inbreeding: yes | 9.95 | |
| | no | 10.03 | 0.83 |
| Weight after hibernation (g) | year: 1 | 10.47 | |
| | 2 | 9.21 | 0.0001 |
| | laying pair: unilateral | 10.31 | |
| | bilateral | 9.37 | 0.0019 |
| Relative loss of weight during hibernation (%) | year: 1 | 20.01 | |
| | 2 | 22.3 | 0.0002 |
| | laying pair: unilateral | 21.08 | |
| | bilateral | 21.23 | 0.84 |

**Table VI.** Estimates of $\sigma_f/\sigma_e$ ratios $\pm$ standard error for random effects (pair model / animal model).

| *Traits* | *Reciprocal effect* | *Box effect* |
|---|---|---|
| Adult age | $0.27 \pm 0.06$ / 0.34 | $0.29 \pm 0.03$ / 0.29 |
| Adult weight | $0.55 \pm 0.05$ / 0.88 | $0.29 \pm 0.02$ / 0.39 |
| Weight after hibernation | $0.56 \pm 0.07$ / 0.81 | – |
| Loss of weight during hibernation | $0.30 \pm 0.05$ / 0.39 | – |

**Table VII.** Estimates of heritabilities and genetic correlations (± SE).

| | *Adult age* | *Adult weight* | *Weight after hibernation* | *Relative loss of weight during hibernation* |
|---|---|---|---|---|
| Adult age | 0.40 ± 0.05<br>0.36 ± 0.07<br>0.38 ± 0.08 | 0.003 ± 0.07 | 0.06 ± 0.12 | − 0.40 ± 0.08 |
| Adult weight | | 0.48 ± 0.04<br>0.60 ± 0.07<br>0.40 ± 0.08 | 0.97 ± 0.01 | 0.59 ± 0.10 |
| Weight after hibernation | | | 0.40 ± 0.05<br>0.54 ± 0.10<br>0.24 ± 0.09 | 0.50 ± 0.16 |
| Relative loss of weight during hibernation | | | | 0.12 ± 0.03<br>0.21 ± 0.06<br>0.03 ± 0.07 |

First line: from animal model; second line: from pair model; third line: from regression.

## DISCUSSION

### *Basic statistics and distributions*

Mean adult age (12 weeks) appeared to be lower than commonly observed in snail farms, ie, 20 weeks (Bonnet et al, 1990). This can be mainly explained by the low population density used in our experiment, so density was probably not a limiting factor as it can be at higher values (Herzberg, 1965; Dan and Bailey, 1982). Lower adult age may also have resulted from optimal environmental conditions.

The distribution of adult age was skewed to the right. Indeed some minimum time is needed before growth is completed. On the other hand, some of the few snails that developed late could be sick or dominated animals. But in this low density situation, competition between snails should not have been very important and the detection of sick animals was impossible as snail pathology is poorly known.

The distribution of relative loss of weight showed an excess of observations near the mean, compared with normality. Therefore, this trait seemed to be relatively constant under our environmental conditions. But it had a large coefficient of variation, because a few extreme individuals contributed to increase the variance.

Results concerning the importance of environmental effects and genetic parameters were similar for the initial or transformed variables. Therefore, the methods used seem relatively robust, with little departure from normality as reported previously by Besbes et al (1993).

### *Correlations*

Adult weight and adult age were very poorly correlated, ie, the decision of stopping the growth was linearly independent of the adult weight. Other simple non-linear

functions (quadratic, reciprocal) were tested but none of them was found to give a better description of the relationship. Bride and Gomot (1991) mentioned that the first snails reaching adult size were bigger but their study did not provide any estimate of this correlation. The present low density, however, could have modified the relationships between adult weight and age.

The correlations between adult age and the two traits measured after hibernation (WAH and RLWDH) showed that the older a snail when it became adult, the higher the WAH and the lower the RLWDH. This is surprising because adult age was not significantly correlated to adult weight which was highly correlated to WAH. We can assume that late animals had lower water contents and then lost less weight during hibernation. The decrease of these correlations after correcting for hibernation duration (see paragraph *Material and methods - Statistical analyses*) shows that they were partially due to the hibernation duration: the older a snail when it became adult, the shorter its hibernation duration and, therefore, the smaller its RLWDH and the higher its WAH.

### *Reciprocal and unilateral/bilateral effects involved in observed variability of traits*

This study confirmed the previous results obtained on reciprocal and unilateral/bilateral laying-pair effects on weight (Dupont-Nivet et al, 1997). The relative magnitude of the reciprocal effect was higher in the animal model because information from the relationship matrix induces a decrease in the residual variance. In previous works, the reciprocal effect was found to be not significant (Murray and Clarke, 1968) or small (Albuquerque de Matos and Serra, 1988), yet these authors used small data sets for estimations. The question asked was how could a snail enhance the growth of its offspring. Since there is no contact between the layer and its offspring, it is likely that the effect is passed on through the eggs. Bride and Gomot (1991) showed that, often in pairs of mating snails, one partner (type I snail) has a very developed albumen gland and spermoviduct while the other (type II snail) has twice as small an albumen gland and a three times as small spermoviduct. Since the vitellus is made in the albumen gland, differences in vitellus quantity and quality in the two snail types may provide explanations for the reciprocal effects. Egg mean weight and adult weight were significantly correlated ($r = 0.29$, $P < 0.01$, $N = 8483$). In order to know if the amount of vitellus could have an effect on offspring growth, an analysis with the egg mean weight as a covariable was made, but the magnitude of the reciprocal effect was not significantly affected. It might be more informative to consider the vitellus quality. The reciprocal effect on other traits such as the hatching rate or early survival rate could also provide interesting information. Bride and Gomot (1991) also assumed (but this is to be confirmed) that type II snails could evolve into type I snails and could remate before laying. Therefore, studying the evolution of reciprocal effect with the egg-laying rank would be useful to know whether the reciprocal effect is partly under genetic control or only under environmental control. More generally, better knowledge concerning reproduction and physiology should be helpful.

If the reciprocal and unilateral/bilateral effects were confirmed and if they had a genetic determinism, it could be interesting to create 'female' lines, selected

for direct and maternal effects, and a 'male' line selected only for direct effects. Crossbreeds between these two lines would then constitute the commercial end product.

### *Additive genetic effects*

Adult shell diameter and adult weight were highly correlated (Dupont-Nivet et al, 1997). Therefore, the estimate of heritability of adult weight was consistent with those already published for *H aspersa* (Albuquerque de Matos and Serra, 1988; Dupont-Nivet et al, 1997) or for close species (Cook, 1965, 1967; Murray and Clarke, 1968). However, Panella (1982) found a lower estimate of the shell diameter heritability ($0.16 \pm 0.06$).

It is worth noting that our experiments took place in a monitored environment (70 snails/$m^2$ at the begining). In conventional snail farming, the part of the phenotypic variance due to environment (in particular, density) might be more important. Dan and Bailey (1982) found that for 143 snails per $m^2$ (which is not a high density in snail farming), young *H aspersa* already showed reduced activity associated with lower feeding and growth. Heritabilities could have been quite different at normal densities where competition becomes critical. Further research is required to investigate possible genotype–environment interaction.

REML estimation with an animal model has been used commonly because of its optimal statistical properties, but it is based on more or less restrictive assumptions, such as a purely additive genetic model which may be not fulfilled here.

Large differences between heritability estimates were obtained according to the estimation method, except for adult age. Differences between the estimates obtained from the animal model or from classical models have already been reported by Visscher and Thompson (1992). Heritabilities estimated from covariances between full-sibs are overestimated since they include dominance and epistatic effects, and a fraction of common environmental effects (see below). However, heritabilities from regression are not biased by dominance, nor by reciprocal effects, but assumed that pairs were not related. But, parents and offspring are raised in different environments, so these estimates may be somewhat low. Heritabilities from animal models may be somewhat biased upwards because of dominance but take all available relationships into account, and were intermediate between previous estimates.

Thus, for all traits except adult age, some non-additive genetic effects might be present.

### *Non-additive genetic effects*

The inbreeding effect is not significant for adult weight ($P = 0.83$). The inbreeding effect was significant for adult age ($P = 0.04$), yet it was not highly significant despite the large number of individuals. This would be consistent with our high heritability estimates (Minvielle, 1990). Moreover, Albuquerque de Matos and Serra (1988) had to produce three generations of full-sib matings before observing an effect of inbreeding on adult weight.

However, heritability estimates for adult weight from pair models and from regression differed, which suggests the existence of some dominance effects. Such a

result was at variance with the previous one because several theories link inbreeding, heterosis and dominance (Minvielle, 1990). Consequently, differences in heritability estimates for adult weight might result from common environmental effects. The reciprocal effect introduced in our model might have been partly unable to cancel out these effects. Further studies on inbreeding and dominance effects will require well-designed experiments such as a diallele experiment involving inbred lines obtained by successive matings between related animals.

### *Genetic correlations*

The genetic correlation between adult age and adult weight was not significantly different from zero, which was consistent with the phenotypic correlation. Theoretically, adult weight is linked to the growth rate and to the duration of growth but it appeared here that weight mainly depended on growth rate.

The genetic correlation between weight after hibernation and loss of weight during hibernation was positive in contrast to the phenotypic correlation. In selection on adult weight, this correlation would need to be taken into account and the correlated evolution of loss of weight during hibernation to be checked.

## CONCLUSION

To increase breeding profitability, the first traits to be improved are adult weight and age. Improvement of adult weight will increase yields and provide snails better suited to market. Decreasing adult age will increase the turnover of animals and reduce the amount of work needed.

Though some heritability estimates were still affected by substantial standard errors, results clearly show that growth traits are highly heritable. In addition, phenotypic variation was high. Therefore, a significant improvement in adult weight and age could be obtained by selection. Since no significant genetic correlation between adult age and adult weight exists, both traits could be easily improved simultaneously using a multi-trait index with appropriate weights depending on their economic importance. However, the knowledge of genetic parameters still needs to be improved. Further experiments to study inbreeding, heterosis, dominance and reciprocal effects are required. A better knowledge of snail biology would also help to guide and interpret the genetic studies, especially to understand maternal effects. Accurate studies of competition phenomena and diseases could also be helpful.

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