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VARIABILITY OF THE GENITAL SYSTEM OF *HELIX POMATIA* L., 1758 AND *H. LUCORUM* L., 1758 (GASTROPODA: STYLOMMATOPHORA)

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ABSTRACT

This paper examines the variability of the genital characters of *Helix pomatia* and *H. lucorum*. Some of these characters are commonly used to distinguish the two closely related species. Variations within and between populations are also investigated. The high variability of these characters can frequently lead to identification errors, especially in eastern Europe. Correct identification of these edible snails is also of commercial importance, different species having a different price. The reciprocal nature of the male parts of the genitalia and the bursa stalk and diverticulum complex is pointed out. A possible function of the diverticulum is suggested on the basis of size relations between genital system features.

INTRODUCTION

The old problem of separating the two related species *Helix pomatia* L., 1758 and *H. lucorum* L., 1758 is not only of academic interest. The world market of these edible snails exceeds 50.10^6 EURO/year. Different taxa fetch very different prices, so distributors and control agencies (such as the French Service de la Répression des Fraudes) need efficient guidelines to discriminate *H. pomatia* from *H. aspersa* Müller, 1774 and from other *Helix* species. All *Helix* species also have to be distinguished from other groups (e.g. the Achatinidae).

Analysis of isozymes indicates that *H. pomatia* and *H. lucorum* are distinct species; the shells, although very variable, can be fully separated by adequate morphometric discriminants (Van Osselaer, Backeljau & Tursch**,** *in preparation*). But these methods do not always solve the practical problem of identification. Identification poses different problems at different steps. First buyers and processing plants have at their disposal the whole snail (shell and fresh animal). Further along the chain, customs can inspect only the cooked meat of animals with much of the visceral mass removed (the hepatopancreas, sometimes the proximal part of the genital apparatus and even some other organs, depending on the extent of the cut). After final processing, shell characters cannot be used anymore, because the meat of the snails very often masquerades in the shell of other *Helix* species (there is no legislation on the use of the shell).

Government-approved identification criteria are largely (if not exclusively) based on the genital system (Nerva, 1987; Biton, 1992). Characters of the reproductive system are indeed traditionally utilised for the systematics (and phylogenetic inference) of pulmonates. Genital system features are commonly used to distinguish *Helix* species (Hesse, 1920; Cesari, 1978; Chevallier, 1980; Grossu, 1983). The size of the diverticulum of the bursa copulatrix—the function of this diverticulum is still unknown (Tompa, 1984; Madec & Guiller,1994)—has been reported (Adam, 1960; Cesari, 1978; Grossu, 1983) to be an effective criterion for distinguishing *H. pomatia* from *H. lucorum*: the organ is long in *H. lucorum*, short or entirely missing in *H. pomatia*. In a mixed sample of the two species, specimens without a diverticulum can be safely attributed to *H. pomatia*, but is a 'short' diverticulum an operational discriminant for the remainder of the specimens? Nerva (1987: 80) has already reported that 12 % of *H. pomatia* in France have a diverticulum longer than 3 mm. Hesse (1920) found specimens of *H. pomatia* having a diverticulum of 2.5 to 9.5 mm in former Yugoslavia. For *H. aspersa*, the length of the diverticulum is the genital system measurement with the highest coefficient of variation and it was reported to have a high discriminating power between populations (Madec & Guiller, 1994).

The reliability of such criteria depends on their variability, especially if one has to identify

specimens collected at different times and in different environments. In spite of the demonstration of seasonal, environmental or physiological variations of reproductive organs in several land snail species (see for example Solem & Christensen, 1984; Emberton, 1985; Bride & Gomot, 1991; Marcos, 1992; Schileyko, Baminger & Stattmann, 1997), the only studies of the variation of the genital system in the genus *Helix* deal with *H. aspersa* (Madec & Guiller, 1993, 1994).

The present study is a biometrical investigation of the variation, and an evaluation of the reliability, of some genital characters of the two closely related species *H. pomatia* and *H. lucorum*.

MATERIALS AND METHODS

Specimens

Specimens were collected from populations covering a great part of the distribution area of *H. pomatia* and *H. lucorum* (Fig. 1). The populations were quite homogeneous, the specimens having been collected from small areas without noticeable gaps in the snails distribution. Some samples were collected in the spring, others in autumn in order to include seasonal variations. Samples are characterised as follows: nearest inhabited locality (Country, code name, month and year of collection, number of specimens measured).

Helix lucorum (13 populations, n = 59 specimens): Harmanli (Bulgaria, Bu1, 05/96, n = 5), Kila (Greece, Gr5, 10/95, n = 5), Sades (Greece, Gr7, 10/95, n = 5), Paradissos (Greece, Gr10, 10/95, n = 5), Lonato (Italy, It1, 10/95 , n = 1), Ferrara (Italy, It3, 10/95, n = 5), Fiero a Seve (It15, 10/95, n = 4), Constanta (Romania, Ro12, 06/95, n = 4), Iasi (Romania, Ro13, 06/95, n = 4), Iasi (Romania, Ro14, 06/95, n = 5), Afyon (Turkey, Tu1, 05/96, n = 5), Inönü (Turkey, Tu2, 05/96, n = 5), Taspinar (Turkey, Tu4, 05/96, n = 5).

Helix pomatia (15 populations, n = 79): Chairière (Belgium, Be3, 07/96, n = 5), Veliko Tarnovo (Bulgaria, Bu3, 06/96, n = 6), Dol (Bulgaria, Bu4, 06/96, n = 5), Gambsheim (France, Fr1, 07/96, n = 5), Görlitz (Germany, Ge1, 05/95, n = 5), Gÿor (Hungary,

Figure 1. Geographic distribution of the samples. Black circles: *H. pomatia.* Black triangles (and italic text): *H. lucorum*. Accurate localities are given in 'Material and Methods'.

Hu1, 06/96, n = 5), Lonato (Italy, It1, 10/95 and 05/96, n = 5), Boara Pisani (Italy, It22, 05/96, n = 5), Rimnicu Vilcea (Romania, Ro6, 06/95, n = 5), Rimnicu Vilcea (Romania, Ro7, 06/95, n = 5), Gheorgheni (Romania, Ro19, 06/95, n = 6), Mosna (Romania, Ro21, 06/95, n = 6), Pascruri (Romania, Ro30, 06/95, n = 6), Wielichowo (Poland, Pol1, 05/96, n = 5), Mickleham (United Kingdom, UK1, 05/97, n = 5).

The specific identity of each of the populations was unambiguously determined by methods (isozymes analysis and shell morphometry) to be described in a separate publication (Van Osselaer *et al.*, *in preparation*).

Live animals brought to the laboratory were reactivated for at least four hours before being killed by several days exposure to –20°C, then stored at –80°C. Specimens were thawed out prior to dissection. Genitalia were then stored in alcohol (70%) for at least one month before measurement. Only fully mature specimens (shell with a reflected lip) were examined.

Genital system measurements

Various parts of the genital system were measured in water, in a Petri dish placed on graph paper. Organs were drawn out with tweezers until extended, without any convolution (under slight tension). All lengths were then measured to the nearest 0.5 mm. The measurements (mostly corresponding to those of Madec & Guiller, 1993, 1994) are depicted in Figure 2.

- BCL1: length of the proximal part of the bursa copulatrix duct (from the bursa to the junction of the diverticulum).
- BCL2: length of the distal part of the bursa stalk (from the junction of the diverticulum to the junction of the stalk to the oviduct).
- BCL12: length of the bursa stalk (from the bursa to the junction of the stalk to the oviduct) for specimens with no diverticulum; BCL12 is BCL1+BCL2 for the other specimens.
- DIVL: length of the bursa copulatrix diverticulum.
- DSL: length of the dart sac.
- EPIL: length of the proximal part of the epiphallus (from the penial retractor muscle to the junction of the flagellum to the vas deferens).
- FLAL: length of the flagellum (from the tip to the junction of the flagellum to the vas deferens).
- PENL: length of the penis (from genital atrium to the penial retractor muscle).
- PRL: length of the penial retractor muscle.
- VAGL: distance from the junction dart sac/vagina to the junction bursa stalk/oviduct-vagina.
- VDL: length of the vas deferens.

The precision and the reproducibility of the measurements of such soft, flexible objects were tested by having the same observer effecting ten repeats (at intervals of several months) on one specimen of *H.*

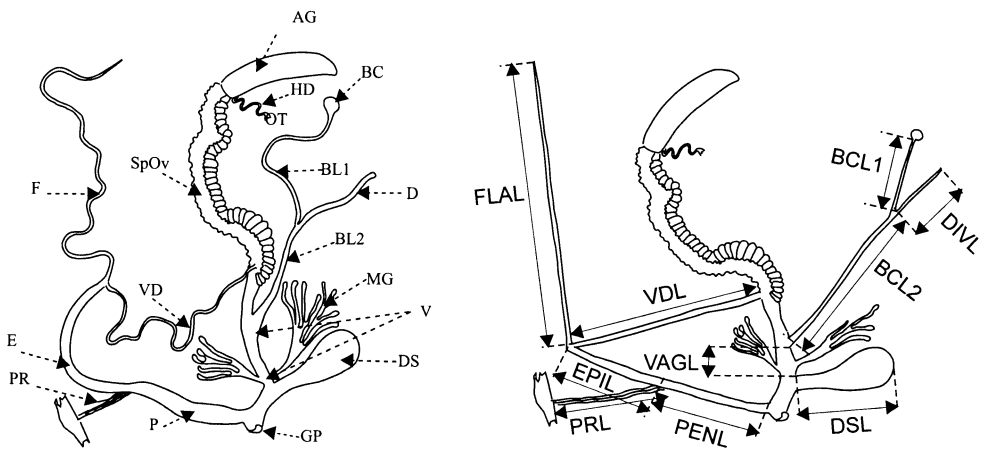

Figure 2.
Left. Schematic drawing of an *Helix* reproductive system. **AG**: albumen gland; **BC**: bursa copulatrix (also called gametolytic gland); **BL1** and **BL2**: proximal and distal part of the bursa copulatrix stalk; **D**: diverticulum; **DS**: dart sac; **E**: epiphallus; **F**: flagellum; **GP**: genital pore; **HD**: hermaphrodite duct; **MG**: multifid glands; **OT**: ovotestis; **P**: penis; **PR**: retractor muscle of the penis; **SpOv**: spermoviduct; **V**: vagina; **VD**: vas deferens.
Right. Genital system measurements. **BCL1**: length of the proximal part of the bursa copulatrix stalk; **BCL2**: length of the distal part of the bursa stalk; **DIVL**: length of the bursa copulatrix diverticulum; **DSL**: length of the dart sac; **EPIL**: length of the proximal part of the epiphallus (from the penial retractor muscle to the flagellum/vas deferens junction); **FLAL**: length of the flagellum; **PENL**: length of the penis; **PRL**: length of the penial retractor muscle; **VAGL**: length of a part of the vagina: from the free oviduct/bursa copulatrix duct junction to the penioviduct angle; **VDL**: length of the vas deferens.

pomatia and one of *H. lucorum* (see Table 1). The precision (St. Dev., see Table 1) of measurements is better than 1 mm but the maximum deviation is about 2–3 mm. This is most probably due to non-reproducibility in the disposition (extension) of organs, prior to measurement.

Shell measurements

Two shell measurements, the length (L) and the diameter (D) as defined by Cox (1960, cited by Kohn & Riggs, 1975) (see Fig. 3) were taken to the nearest 0.05 mm with a digital display calliper. The reproducibility of the measurements (see Table 1) was tested by effecting ten repeats (at intervals of several months) on one specimen of *H. pomatia* and one of *H. lucorum*.

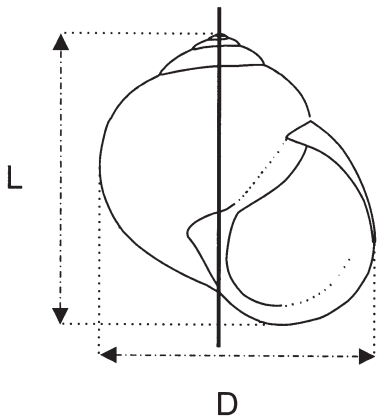

Figure 3. Schematic draw of a *Helix* shell. The length (**L**) and the diameter (D) are defined perpendicular and parallel to the coiling axis.

Data analysis

Analyses were effected on either raw measurements, ratios of raw measurements, or ratios of measurements to shell size (L or D). Because several specimens of *H. pomatia* had no diverticulum, two different sets of data were utilised:

Data Set A includes BCL1 and BCL2. It cannot be used for specimens without diverticulum (which are known to be *H. pomatia* and in which BCL1 and BCL2 cannot be determined).

Data Set B includes all specimens and uses BCL12 (instead of BCL1 and BCL2). Specimens without diverticulum are given a null value for DIVL. All statistical analyses were performed with the program STATISTICA (5.1).

RESULTS

Correlations

The mean shell length (L) was not significantly different for our samples of the two species (*H. pomatia*: 38.80 mm; *H. lucorum*: 37.96 mm, t-test: $p = 0.18$, not significant). In contrast, the mean diameter (D) differed statistically (*H. pomatia*: 37.81 mm; *H. lucorum*: 40.29 mm, t-test: $p<0.001$) but the overlap of distributions was too large to allow clear-cut separation.

No important correlation was found between genital characters measurements (maximum: $r = 0.72$, see Table 2). Genitalia measurements of specimens of *H. pomatia* or *H. lucorum* showed no substantial (maximum: $r = 0.61$, see Table 2) correlation with the shell measurements L and D, suggesting that the genital measurements vary independently of the shell

Table 1. Precision and reproducibility of measurements. Ten repeats of measurements of genitalia and shell on one specimen of each species. (see Materials and Methods). C.V. is the coefficient of variation (this is the standard deviation expressed as a percentage of the mean = St. Dev. * 100/Mean).

	FLAL	EPIL	PRL	PENL	DSL	VDL	BCL2	BCL1	DIVL	VAGL	L	D
H. lucorum JD-0768												
Min. (mm)	68.0	6.0	6.5	17.0	9.0	34.0	29.0	41.0	22.0	9.0	41.17	45.80
Max. (mm)	71.0	8.0	8.5	20.5	11.0	36.0	33.0	42.0	25.0	11.5	41.39	46.00
Max.–Min. (mm)	3.0	2.0	2.0	3.5	2.0	2.0	4.0	1.0	3.0	2.5	0.22	0.20
Mean (mm)	70.4	6.9	7.7	18.9	10.4	35.0	30.5	41.5	23.9	10.2	41.23	45.90
St. Dev. (mm)	0.89	0.66	0.68	1.18	0.58	0.74	1.38	0.42	0.77	0.72	0.06	0.06
C.V. (%)	1.26	9.61	8.81	6.24	5.61	2.12	4.52	1.00	3.21	6.98	0.15	0.13
H. pomatia JD-0368												
Min. (mm)	56.0	5.0	17.0	16.5	7.0	28.0	26.0	29.0	14.0	7.0	32.38	31.15
Max. (mm)	58.0	6.0	19.0	18.0	7.5	31.0	28.0	30.5	16.0	8.0	32.55	31.32
Max.–Min. (mm)	2.0	1.0	2.0	1.5	0.5	3.0	2.0	1.5	2.0	1.0	0.17	0.17
Mean (mm)	57.3	5.5	18.00	17.2	7.2	29.9	27.2	29.9	15.0	7.1	32.48	31.20
St. Dev. (mm)	0.74	0.35	0.63	0.51	0.25	0.85	0.60	0.42	0.52	0.32	0.05	0.05
C.V. (%)	1.30	6.31	3.51	2.96	3.45	2.84	2.21	1.39	3.47	4.48	0.16	0.16

Table 2. Coefficients of correlation between measurements. (1): *H. pomatia*, (2): *H. lucorum*. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

r	FLAL	EPIL	PRL	PENL	DSL	VDL	BCL2	BCL1	DIVL	VAGL	L
EPIL (1)	0.21										
EPIL (2)	–0.09										
PRL (1)	0.16	0.10									
PRL (2)	–0.11	–0.16									
PENL (1)	0.43***	0.42***	0.22								
PENL (2)	0.13	0.03	0.04								
DSL (1)	0.32**	0.01	0.26*	0.23*							
DSL (2)	0.30*	0.27*	–0.24	0.51***							
VDL (1)	0.47***	0.22*	0.27*	0.67***	0.27*						
VDL (2)	0.30*	0.08	–0.03	0.49***	0.38**						
BCL2 (1)	0.27	0.20	0.29*	0.40**	0.38**	0.59***					
BCL2 (2)	0.29*	0.14	–0.008	0.59***	0.46***	0.72***					
BCL1 (1)	–0.03	–0.14	0.05	–0.04	0.18	0.15	–0.09				
BCL1 (2)	0.11	0.14	0.10	0.28*	0.12	0.57***	0.42***				
DIVL (1)	–0.15	–0.27*	0.12	–0.27*	–0.04	–0.13	–0.25	0.64***			
DIVL (2)	0.05	0.25	–0.04	0.38**	0.21	0.57**	0.53***	0.72***			
VAGL (1)	0.17	0.12	0.08	0.22*	0.18	0.29**	0.34*	0.35*	0.11		
VAGL (2)	0.07	0.38**	–0.0009	0.45***	0.47***	0.44***	0.50***	0.32*	0.31*		
L (1)	0.36***	0.11	0.17	0.44***	0.34**	0.53***	0.48***	0.29*	–0.06	0.30**	
L (2)	0.42***	0.13	–0.08	0.25	0.30*	0.48***	0.61***	0.38**	0.42***	0.23	
D (1)	0.31**	0.04	–0.03	0.36***	0.29**	0.37***	0.35**	0.20	–0.07	0.17	0.77***
D (2)	0.15	0.06	0.08	0.21	0.24	0.41***	0.43***	0.56***	0.57***	0.28*	0.75***

size. In all cases, the possibility of non linear correlation was considered but none was found.

Discrimination power of genital system measurements

Does the genital system allow distinguishing unambiguously *H. pomatia* from *H. lucorum*, as it is commonly admitted (Cesari, 1978; Chevallier, 1980; Grossu, 1983) ?

The mean values of FLAL, EPIL, DSL, VDL, BCL1, BCL2, DIVL, VAGL were statistically different for the two species (t-test, $p < 0.001$ excepted for VDL for which $p < 0.01$) (see Table 3). But for all these variables, the overlap of the values was always considerable, as shown by the standard deviations (see Table 3). Similar results were obtained if the variables were size-standardised by dividing them by a shell measurement (not illustrated).

Several multivariate analyses by PCA and UPGMA (these methods do not postulate *a priori* categories) were performed on raw genitalia data, ratios of raw data, raw data divided by the length or the diameter of the shell and even on mixtures of raw data and ratios. All attempts gave similar, inconclusive results. In the best cases, the membership of many specimens was unclear.

To distinguish *H. lucorum* from *H. pomatia*, Biton (1992) has proposed the following discriminant equation (with standardised coefficients):

$$Y = 0.495 * BCL1/BCL2 + 0.547 * DIVL + 0.180 * EPIL$$

where $Y < 0$ for *H. pomatia* and $Y > 0$ for *H. lucorum*. The function can be applied only to the specimens of *H. pomatia* which possess a diverticulum (BCL1 and BCL2 cannot be determined on the others).

When applied to our sample, this equation did indeed give a bimodal distribution (see Fig. 4). If one checked the fate of previously identified snails, 5 (9.61 %) of 52 incorporated specimens of *H. pomatia* (those having a diverticulum) and 5 (8.62 %) of 58 specimens of *H. lucorum* (the single specimen from locality It1 was discarded in the multivariate analyses) were incorrectly classified. But if one should use the equation to separate unidentified snails, then all specimens in the zone of overlap will remain of doubtful status. These amounted to 54.54 % of the total number of specimens (for details, see Table 4). These calculations were made on individual values and not on the classes of the histogram (Fig. 4), to make the overlap insensitive to the number of classes. This percentage is similar to that observed in the histogram given by Biton (1992).

It was noticed that the root proposed by Biton (1992) was highly correlated ($r^2 = 78.55$ %, $p < 0.0001$) with the length of the diverticulum (which has the largest standardised coefficient).

Using only the length of the diverticulum, the overlap zone for DIVL was constituted by 39.09 % of the total number of specimens (for details, see Table 4) (in the frequency histogram of DIVL [see Fig. 5], one specimen *H. pomatia* [DIVL = 24 mm] appeared to be aberrant and has been disregarded for the calculation of the

Table 3. Comparison of genital system measurements. (1): *H. pomatia*, (2): *H. lucorum*. DIVL(a) incorporates all specimens of *H. pomatia* and null values are given to specimens lacking a diverticulum. DIVL(b) incorporates only the specimens of *H. pomatia* possessing a diverticulum. ***: = $p < 0.001$, **: = $p < 0.01$, N.S.: not significant. C.V. is the coefficient of variation.

	Mean (mm)		t	p	n		St. Dev. (mm)		CV (%)	
	(1)	(2)			(1)	(2)	(1)	(2)	(1)	(2)
FLAL	64.15	71.78	–3.61	***	79	59	9.57	15.20	14.92	21.17
EPIL	5.77	6.95	–4.42	***	79	59	1.34	1.79	23.22	25.75
PRL	11.92	14.12	–1.79	N.S.	76	51	4.80	8.98	40.27	63.60
PENL	19.68	18.86	1.53	N.S.	79	59	3.12	3.07	15.85	16.28
DSL	8.57	11.03	–9.64	***	79	59	1.18	1.81	13.77	16.41
VDL	36.59	33.68	2.80	**	79	58	5.78	6.31	15.80	18.73
BCL2	37.54	38.85	–0.72	N.S.	52	59	8.08	10.74	21.52	27.64
BCL1	18.14	29.91	–9.80	***	52	59	6.02	6.55	33.19	21.90
BCL12	56.07	68.75	–6.10	***	79	59	9.63	14.76	17.17	21.47
DIVL (a)	3.78	18.18	–16.51	***	79	59	4.62	5.62	122.22	30.91
DIVL (b)	5.74	18.18	–12.65	***	52	59	4.60	5.62	80.14	30.91
VAGL	8.37	10.36	–4.40	***	79	59	2.03	3.27	24.25	31.56

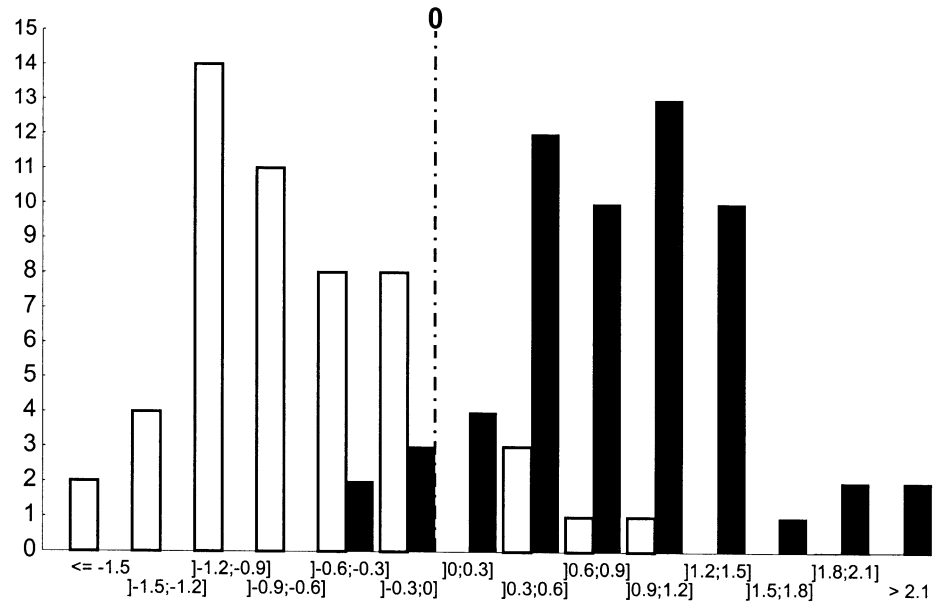

Figure 4. Histogram of the root proposed by Biton (1992) to discriminate *H. pomatia* (white columns) and *H. lucorum* (black columns) and applied to our samples (after centring and reduction). Y = 0.495 * BCL1/BCL2 + 0.547 * DIVL + 0.180 * EPIL (standardised coefficients).

Table 4. Comparison of the performance of different separation methods on specimens with diverticulum.

	% of all specimens in overlap zone	% of all *H. pomatia* in overlap zone	% of all *H. lucorum* in overlap zone
All specimens included			
BITON's equation	54.54	38.46	68.96
Diverticulum alone	64.86	44.23	83.05
Equation (2)	19.01	15.38	22.41
One aberrant specimen of *H. pomatia* excluded			
BITON's equation	42.20	37.25	46.55
Diverticulum alone	39.09	43.14	35.59
Equation (2)	18.35	13.72	22.41

overlap between the two species). On our samples, this measurement alone thus performed at least as well as (and possibly better than) Biton's equation.

When discriminant analyses of raw data were performed on our samples, data sets A and B (see Data analysis) gave similar results and revealed a significant difference between the two species under consideration (Chi-square test, $p<0.001$). Only one root was extracted in all cases submitted to the analysis (this is always the case when discriminating only two groups). With standardised coefficients, it is:

Root = 0.235 * FLAL + 0.181 * EPIL – 0.073 * PENL + 0.524 * DSL – 0.637 * VDL – 0.022 * BCL2 + 0.276 * BCL1 + 0.668 * DIVL – 0.075 * VAGL. (1)

With raw coefficients it is:

Root = 0.018 * FLAL + 0.116 * EPIL – 0.024 * PENL + 0.333 * DSL – 0.105 * VDL – 0.002 * BCL2 + 0.044 * BCL1 + 0.129 * DIVL – 0.028 * VAGL. (2)

Raw coefficients are also given here because they allow comparison with other samples, of

different composition (and possibly with a different barycentre).

If the analysis incorporated the specimens of *H. pomatia* lacking a diverticulum (34.18% of our sample of this species), their null value would of course decrease the mean of DIVL for *H. pomatia* (see Table 3). The distance between the two species was therefore increased for this variable but this did not improve the separation (on overlap or on misclassified specimens). Using BCL12 instead of BCL1 and BCL2 led to a less efficient discrimination (not illustrated).

When applied to our sample, the root of the discriminant based on raw measurements also gave a bimodal distribution (Fig. 6). One should note that the discriminant root (eq. 1) has a 'critical value' (this is the intersection of the fitted normal distributions of the two groups and

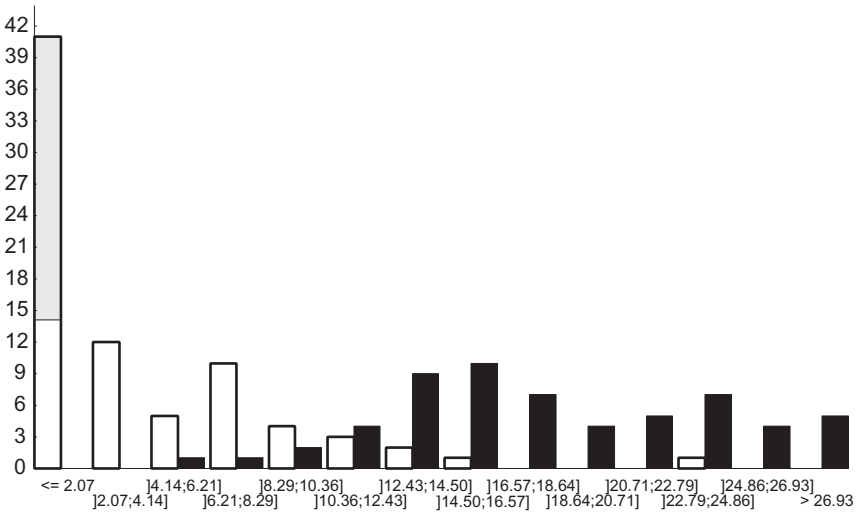

Figure 5. Histogram of the length of the diverticulum (DIVL) of *H. pomatia* (white columns) and *H. lucorum* (black columns). Individuals devoid of diverticulum (DIVL = 0, n = 27) are indicated in grey.

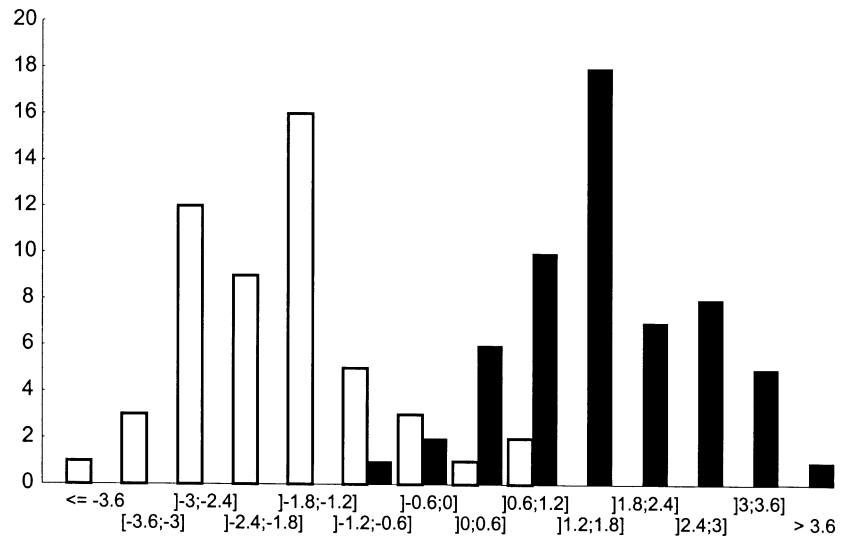

Figure 6. Histogram of the root discriminating *H. pomatia* (white columns) and *H. lucorum* (black columns). Root (standardised coefficients) = 0.235 * FLAL + 0.181 * EPIL – 0.073 * PENL + 0.524 * DSL – 0.637 * VDL – 0.022 * BCL2 + 0.276 * BCL1 + 0.668 * DIVL – 0.075 * VAGL.

is used as the limit for misclassified specimens, see for example Johnson & Wichern, 1988) of – 0.12 (not 0). One misclassified specimen of *H. lucorum* is added if one takes the 'critical value' as 0. If one checked the fate of previously identified snails, then 3 of 52 (5.77 %) incorporated specimens of *H. pomatia* and 2 of 58 (3.45 %) specimens of *H. lucorum* were misclassified. But if one used the equation to separate unidentified snails, all specimens in the zone of overlap remained of doubtful status. These amounted to 19.01 % of the total number of specimens (for details, see Table 4).

These figures were a marked improvement over those obtained by the method of Biton (1992), reported here above. This was probably due to the inclusion of more parameters in the analysis but a fully objective comparison of the two methods was not possible because we are dealing here with a different sample. Equation (2) seems also to be the least sensitive to the presence of aberrant specimens (see Table 4).

The efficiency of the methods (for identifying individual snails) did indeed vary with the origin of the specimens. All the specimens in the overlap zone of the histogram (Fig. 6) originated from countries where both species are present (Greece, Romania, Bulgaria and Italy). 5.88% (5/85) of all specimens from these countries were misclassified. This included 7.15% (3/42) of all *H. pomatia* and 4.65% (2/43) of all *H. lucorum*. The zone of overlap (doubtful cases) included 35.29% of the total sample, consisting of 35.29% (11/42) of all *H. pomatia* and 26.19% (19/43) of all *H. lucorum*. So, a discrimination based upon genital system measurements was not reliable in well over 40% of all cases. This figure was still higher if the sample came only from Romania and Bulgaria. In contrast, if the specimens from Romania and Bulgaria were removed from our sample, separation was total.

As stated above, analyses of individual specimens in our total sample by methods that do not postulate *a priori* categories (PCA, UPGMA) invariably led to largely overlapping groups (such Fig. 7). In contrast, when applied to centroids of populations samples, these methods yielded a clear-cut discrimination (the two species were separated along axis 1, see Fig. 8). This indicated that the problems met in the analyses of individuals snails stemmed from intrapopulation variability (now suppressed). In practice, this approach was restricted to samples representing populations (we have never collected the two species together, with the exception of one single specimen of *H. lucorum* found with a large population of *H. pomatia* at Lonato [It1]).

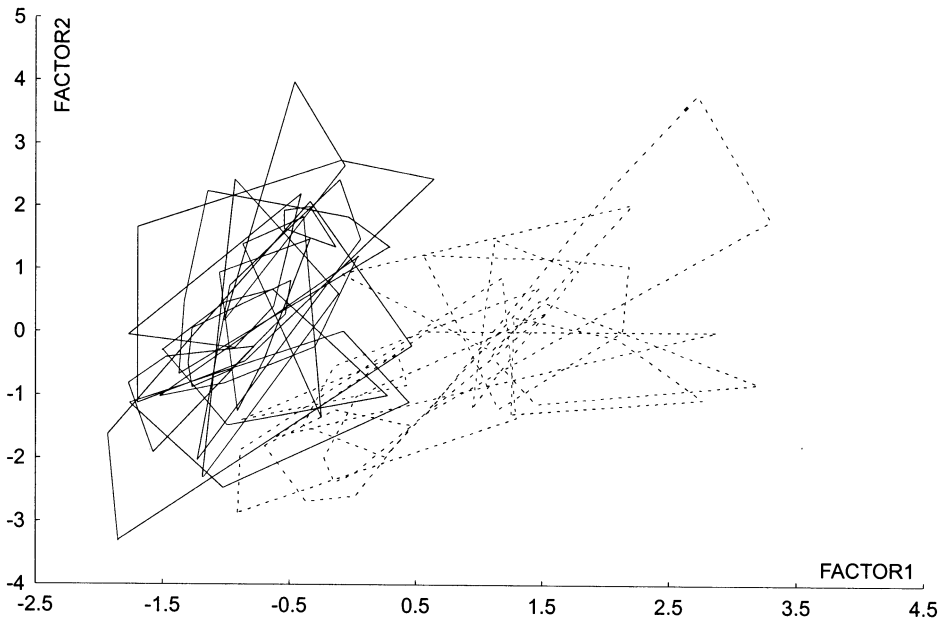

Figure 7. Projection of the individuals of the populations used for the principal component analysis on centroids (see Fig. 8). Minimum convex polygons are given instead of individuals for the sake of clarity. Solid polygons: populations of *H. pomatia*. Dotted polygons: populations of *H. lucorum*.

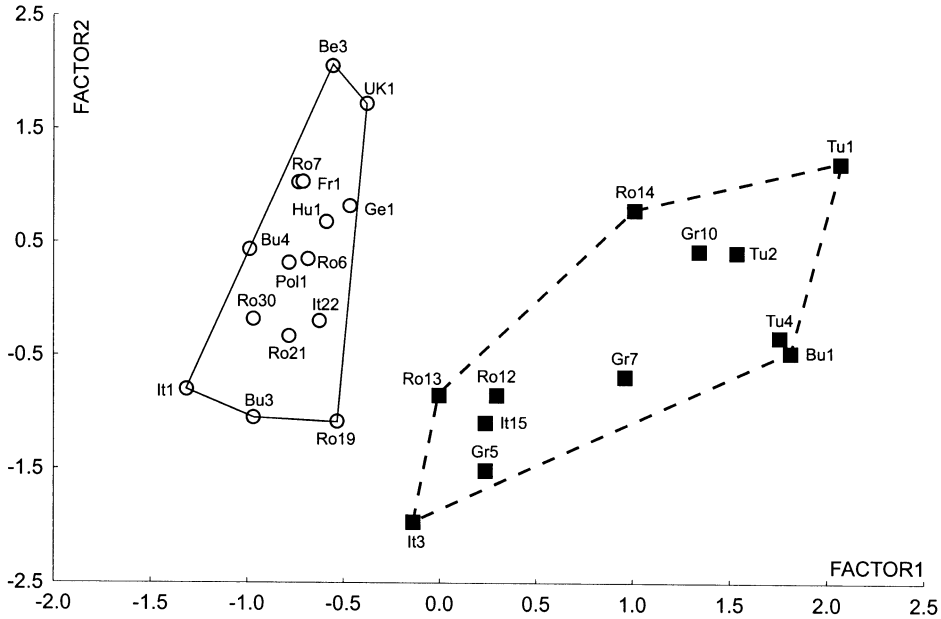

Figure 8. Principal component analysis on centroids of all populations (Varimax). Data set B (see text: Data Analysis). Open circles: *H. pomatia*; Black squares: *H. lucorum*. Variables : FLAL, EPIL, PENL, DSL, VDL, BCL12, DIVL, VAGL. Factor 1 (48.15 % of the total variance) = 0.127 * FLAL + 0.193 * EPIL – 0.014 * PENL + 0.248*DSL – 0.037045*VDL + 0.206 * BCL12 + 0.248 * DIVL + 0.220 * VAGL. Factor 2 (24.66 % of the total variance) = 0.079 * FLAL – 0.017 * EPIL + 0.458 * PENL – 0.044 * DSL + 0.471 * VDL + 0.148 * BCL12 – 0.192 * DIVL + 0.006 * VAGL.

Relative size of genital organs

For all specimens of *H. pomatia* possessing a diverticulum and for all *H. lucorum,* the diverticulum (DIVL) was always shorter than the proximal part of the bursa stalk (BCL1) (Fig. 9). It obviously followed that (BCL2+DIVL) was always shorter than BCL12 (Note: DIVL and BCL1 are amongst the best correlated genital system measurements [*H. pomatia*: r = 0.64; *H. lucorum*: r = 0.71, see Table 1]). Excepted for four specimens, (FLAL+EPIL)—the sum of the lengths of the organs responsible for the formation of the spermatophore (Lind, 1973)—was always longer than (BCL2+DIVL) (Fig. 10). But (FLAL+EPIL) was not always longer than BCL12 (Fig. 11).

Geographic variation of the diverticulum in H. pomatia

The distribution of the diverticulum length (DIVL) for all the populations of *H. pomatia* in our sample is shown in Figure 12. The longer diverticula were found in the Eastern parts of the species distribution.

We have not enough data to demonstrate the existence of a strictly clinal variation but the existence of a geographical trend seemed obvious (see Fig. 13). For unknown reasons, intrapopulation variability of the diverticulum was also much higher in Romania and Bulgaria.

The same results (not illustrated) were obtained if DIVL was standardised by dividing it by the length (L) or the diameter (D) of the shell. The length of the diverticulum of *H. aspers*a also seems to vary according to a geographic pattern (Madec & Guiller, 1994). In their samples, the smaller diverticula are found in the Southern and Eastern parts of the species distribution.

DISCUSSION

Discriminating characters

The problem addressed here is an example of taxonomic decisions having important economical repercussions. French legal texts delimitate four commercial categories of snails, fetching very different prices:

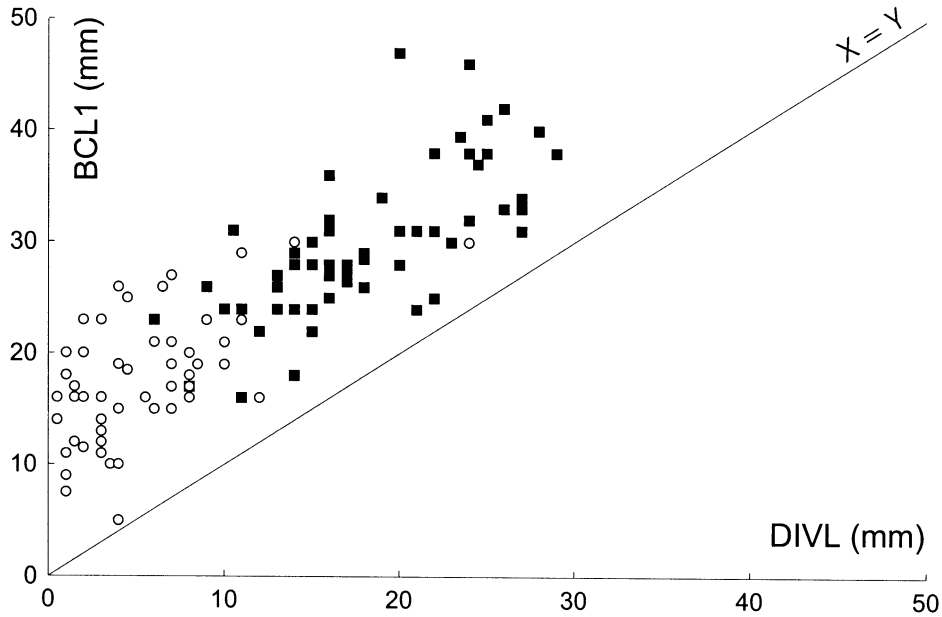

Figure 9. Scatter diagram of BCL1 *vs*. DIVL. Open circles: *H. pomatia*, Black squares: *H. lucorum*. For all specimens DIVL is shorter than BCL1.

1/ *Helix pomatia* products ('escargot de Bourgogne').
2/ *Helix aspersa* products ('petit gris' et 'gros gris').
3/ other *Helix* species, notably *H. lucorum* ('escargot').
4/ various *Achatina* species, still posing many taxonomic problems ('escargot Achatine').

In practice, can these categories be safely recognised on samples of cooked and mutilated snail meat of uncertain origin?

Even if the problem is reduced to the discrimination of *H. pomatia* from *H. lucorum*, measurements of the genital system, although presenting statistically significant differences, do not allow unambiguous identification of specimens, with the exception of the specimens of *H. pomatia* that are devoid of diverticulum. Even with improved discriminant equations, far too many specimens remain of dubious status in the overlap zone.

The problem met by the controlling agencies is much worse than the one we encountered with the sample treated here. The majority of commercial *Helix* come from Eastern European countries and a significant proportion originate from Romania and Bulgaria (where the genitalia of the two species happen to be the less distinct). The commercialised products are often of mixed origin and may contain some others species, not easily discernible by non specialists (or even by specialists). The 'escargot' category is constituted mainly by *H. lucorum*, but may also contain other species such as *H. secernenda* Rossmässler, 1847 or *H. albescens* Rossmässler, 1839 (which could as well be mistaken in good faith for *H. pomatia*). It may also contain several other taxa of as yet questionable taxonomic status (see e.g. Chevallier, 1980).

Worse still, all specimens in our sample are adult snails, uncooked and submitted to identical treatment. Commercial lots are not limited to fully mature specimens. The presence of juvenile snails (*H. lucorum* with a smaller diverticulum) could increase the already sizeable overlap. Several factors are known to affect also the genital measurements (e.g. the retraction of the animal, see Emberton, 1989) and many companies have their own recipes for preparing and cooking the snails. The effects of such differences in processing are not known.

Variability

Great intra-specific variability in phenotypes and/or in allele frequencies has frequently been

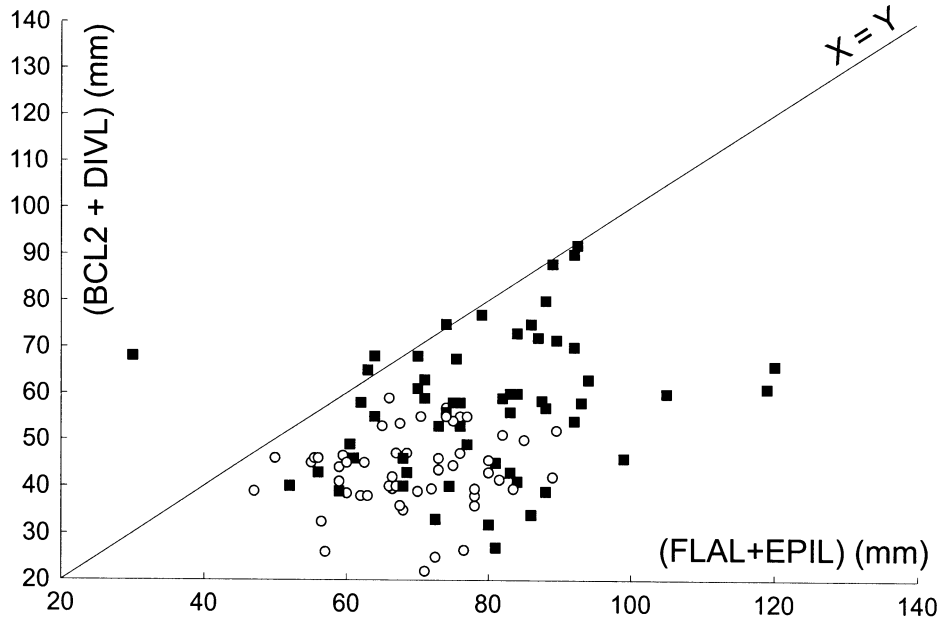

Figure 10. Scatter diagram of (BCL2 + DIVL) *vs*. (FLAL + EPIL). Open circles: *H. pomatia*, Black squares: *H. lucorum*. For nearly all specimens (FLAL + EPIL) is longer than (BCL2 + DIVL).

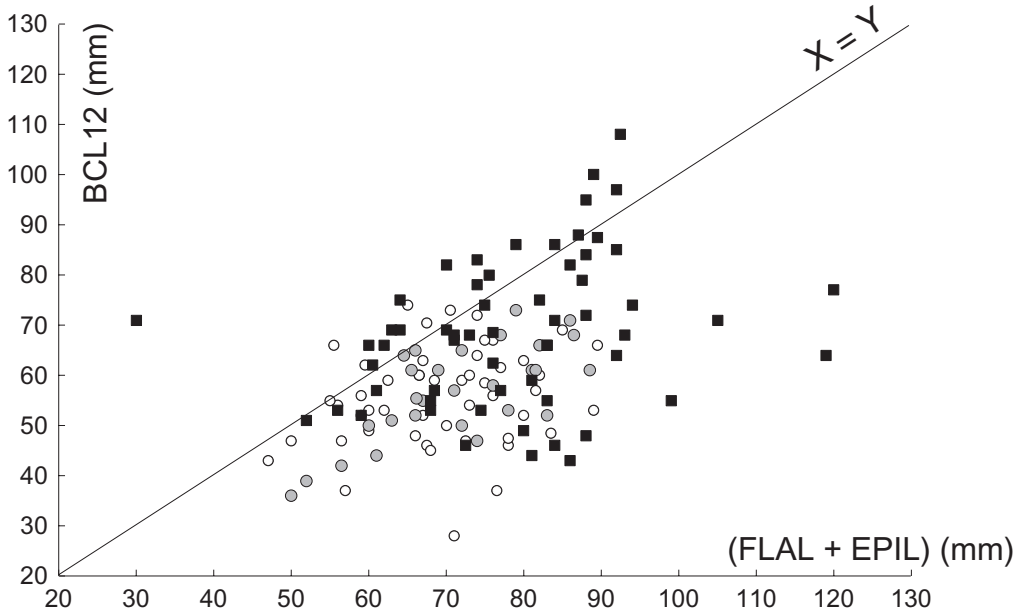

Figure 11. Scatter diagram of BCL12 *vs*. (FLAL+EPIL). Open circles: *H. pomatia* specimens having a diverticulum, Grey circles: *H. pomatia* specimens devoid of diverticulum, Black squares: *H. lucorum*. (FLAL + EPIL) is not always longer than BCL12.

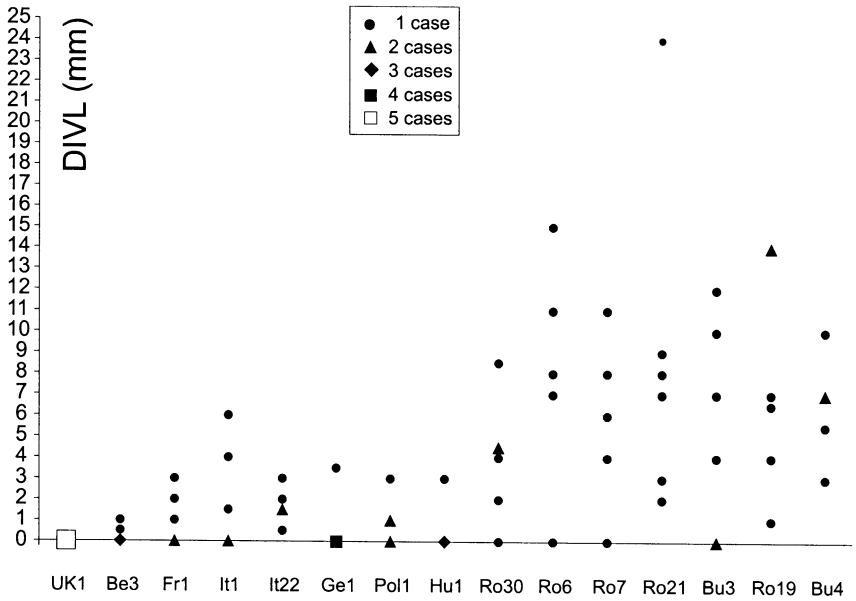

Figure 12. Distribution of the length of the diverticulum in the populations of *H. pomatia*, ordered along a fictional West-East axis.

reported in landsnails. It is even expected that appropriate sets of characters could completely differentiate populations of a same species (Mayr, 1969; Futuyma, 1986; Tursch, 1998) and it is probably the lack of investigations on the variability of the genitalia of *Helix* species (a shadow of 'typological thinking') that has led to the widespread confidence in the use of these organs to distinguish species (Hesse, 1920; Cesari, 1978; Chevallier, 1980; Grossu, 1983).

The data given here show the expected, important intra-population (see Fig. 7) and intra-specific variability of all the genital measurements (see Table 3). Their coefficients of variation are much greater (for both species) than those of the simple shell measurements (*H. pomatia*. H: St. Dev. = 3.06 mm, CV = 7.89 %; D: St. Dev. = 2.66 mm, CV = 7.03 %; *H. lucorum*: H: St. Dev. = 4.26 mm, CV = 11.22 %; D: St. Dev. = 4.02 mm, CV = 9.98 %).

Several papers have reported the high intraspecific variability of the genital organs of landsnails, even within populations (e.g. Backhuys, 1972 for *Theba pisana* Müller, 1774; Tomiyama, 1988 for *Satsuma tanegashimae* (Pilsbry); Outeiro, Mato, Riballo & Rodriguez, 1990 for *Cochlicopa* species; Madec & Guiller, 1994 for *H. aspersa*). This has led to reconsideration of the discriminating power of these characters (Outeiro *et al.*, 1990). Our data point to the necessity of studying more specimens from more localities in order to (re)-evaluate the efficiency of the reproductive organs as taxonomic discriminants in the genus *Helix*.

Hypothesis on the utility of the diverticulum

The name 'diverticulum' is unfortunate because it implies that the organ is an annex of the bursa stalk (which has phylogenetic implications), as it appears in some illustrations depicting the *Helix* genital system (Cesari, 1978; Grossu, 1983). In most cases, the diameter of the diverticulum is actually larger than the diameter of the upper part of the bursa stalk. That the diverticulum is the continuation of the lower part of the bursa duct is very well illustrated by Backhuys (1972), Schileyko (1978), or Varga (1989) and corresponds to our own observations. It is better interpreted as a blind-ended tube (Lawrence, 1995).

The spermatozoa of *H. pomatia* are stored in the head of the spermatophore, in a state of temporary inactivation, during transfer into the vagina of the partner (Lind ,1973). The spermatophore is then pushed up into the bursa stalk, which is the continuation of the vagina (not of the oviduct). Lind (1973) demonstrated that the spermatozoa start leaving the intact spermatophore shortly after reactivation. They

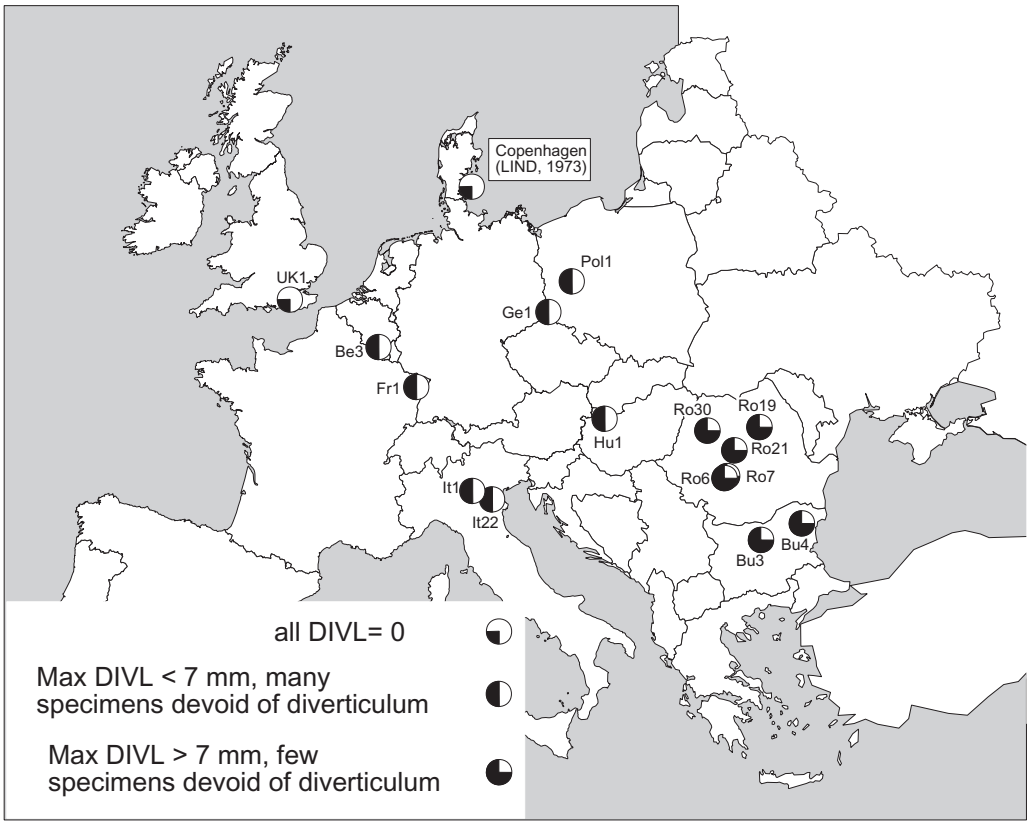

Figure 13. *H. pomatia* samples: distribution of the intra-population variability of DIVL. Data on Copenhagen specimens are from Lind (1973).

escape partly through the tail, partly through the base of the tail but never through the anterior part of the spermatophore. The bursa stalk has strong upward peristaltic motions, leading the contents of the stalk towards the bursa copulatrix where it is destroyed.

In Helicidae, the bursa copulatrix should preferably be called the *gametolytic gland* (Giusti & Andreini, 1988) as it has been shown to digest its content, i.e. excess of auto-sperm, allosperm, spermatophore and parasites (Lind, 1973; Giusti & Andreini, 1988). It is not a storage area as implied by terms such as 'spermatheca' or 'receptaculum seminis', used by several authors (e.g. Miller, 1972; Grossu, 1983; Guerrucci *et al.,* 1988). The stalked pouch, which may primitively have functioned purely as a bursa copulatrix, has become less specific in its function in several gastropods taxa (Bayne, 1974). A terminology based solely on functional relationships cannot be entirely consistent between taxa when homologous structures have altered their function in the course of evolution (Bayne, 1974). The vaguer but familiar term '*bursa copulatrix*' will be widely used here, to follow common usage.

The excellent work of Lind (1973) leads to the conclusion that the spermatophore of *Helix pomatia* is not needed for sperm protection but is absolutely necessary for allowing some of the spermatozoa to escape from the bursa stalk. The particular shape (canal) of the spermatophore tail establishes a connection between the spermatophore body (initially containing the transferred sperm) and the entrance of the oviduct.

The longer the part of the spermatophore's tail that is not rapidly inserted in the bursa stalk, the greater the probability (in terms of time and/or numbers) of having spermatozoa escape destruction in the gametolytic gland and migrate to the oviduct. There are two ways of

achieving this: one is to increase the length of the spermatophore, the other is to shorten the organs in which it is engulfed.

After being inserted into the distal part of the bursa stalk (Fig. 1, BL2), the spermatophore always penetrates into the diverticulum (Fig. 1, D) when this is present (for Helicoidea, see Schileyko, 1978; for *Arianta arbustorum*, see Hofmann, 1923 cited by Lind, 1973). In the absence of a diverticulum, the spermatophore can only continue to migrate into the bursa stalk. As the diverticulum (DIVL) is always shorter than the proximal part of the bursa stalk (Fig. 1, BCL1) (see Fig. 9), we suggest that the diverticulum could serve to increase the probability of efficient spermatozoa transfer by lengthening the portion of the spermatophore's tail protruding from the stalk. Several indications seem to favour this hypothesis.

The above comparisons of the size of organs were made within individual specimens. But *Helix* are cross-fertilising animals, so it would be more relevant to compare the length of the spermatophore to that of the receiving organs (i.e. BCL12 *or* BCL2 + DIVL) for all possible mating pairs in each of the sampled populations.

As a first approximation, the length of the spermatophore can be taken to be (FLAL + EPIL) (see Lind, 1973). This is the sum of the lengths of the epiphallus (responsible for the formation of the head, the neck and the body of the spermatophore, see Lind, 1973) and the flagellum (responsible for the formation of the tail, see Lind, 1973).

H. pomatia.—In our sample of *H. pomatia*, there are 340 possibilities [$\mathrm{Sum}_i(n_i)*(n_i\text{-}1)$, *i* stands for populations] of intrapopulation sperm transfer. (FLAL + EPIL) of the potentially 'giving' partner is not longer than BCL12 of the potentially 'receiving' partner in only 93 (27.35%) of the 340 cases.

One could now have an indication of the influence of the diverticulum by comparing (FLAL + EPIL) to BCL12 (for the combinations involving 'receiving' partners without diverticulum) *or* to BCL2 + DIVL (for the combinations involving receiving partners with diverticulum). In only 26 cases (7.65%) is (FLAL + EPIL) of the 'giving' partner not longer than (BCL2 + DIVL) *or* BCL12 of the 'receiving' partner. Thus the presence of the diverticulum—accounted for by replacing BCL12 by (BCL2 + DIVL)—increases the probability of the spermatophore being longer than the organs in which it is received.

7.65% is probably still too high a figure because the length of the spermatophore may have been underestimated. Indeed, Lind (1973: 55) reported that the length of the spermatophore tail is actually longer (by a factor 1.22) than the flagellum in which it is formed. Based on the data of Lind (1973: 55), the mean length of the spermatophore could be evaluated as ([1.22*FLAL] + EPIL). In this second, refined approximation, only 16 cases (i.e. 4.71 %) still have (1.22*FLAL) + EPIL not longer than BCL12, while all of the 340 cases have ([1.22*FLAL] + EPIL) longer than BCL2 + DIVL *or* BCL12.

H. lucorum.—In our samples of *H. lucorum*, there are 224 possibilities of intrapopulation sperm transfer. In 168 (75.00 %) cases (FLAL + EPIL) of the 'giving' partner is not longer than BCL12 of the 'receiving' partner (all specimens of this species have a diverticulum).

If one compares (FLAL + EPIL) to BCL2 + DIVL, in only 42 cases (18.75 %) (FLAL + EPIL) of the 'giving' partner is not longer than (BCL2 + DIVL) of the 'receiving' partner. Here also the presence of the diverticulum, accounted for by replacing BCL12 by (BCL2 + DIVL), could increase the probability of the spermatophore being longer than the organs in which it is received. No data allow a second approximation of the spermatophore length for this species.

***H. pomatia* without diverticulum.**—If the diverticulum is, in both species, so useful, what happens with the many specimens of *H. pomatia* in which the diverticulum is absent or so reduced as to be useless ? If our hypothesis is correct, the absence (or extreme reduction) of the diverticulum should be compensated, for instance by shortening BCL12 of the specimens without a diverticulum or by increasing the length of the spermatophore of all specimens.

11 of the populations studied here include specimens lacking a diverticulum. For every population, the BCL12 of the specimens lacking the diverticulum is always less than (1.22* FLAL) + EPIL of all the specimens, suggesting that the absence of diverticulum appears to be compensated for by a reduction of BCL12. No statistical test could be however performed, as each of the samples was too small.

The above observations are in agreement with the generalisation of Schileyko (1978, translated by Y. Kantor): '. . . it is possible to say that in species with long diverticulum, the long flagellum is always present, but not all species with long flagellum are obliged to have the diverticulum of the corresponding length'. The relative sizes of the spermatophore (and

hence the flagellum and the epiphallus), the bursa stalk and the diverticulum could act as a barrier to successful reproduction between individuals if the spermatophore was the 'wrong' size for the recipient organs (Lace, 1992).

The lack of important correlations between genital characters and shell size (see Results: Correlations) seems also to suggest that breeding would not be prevented between animals of different shell sizes.

Our interpretation is not necessarily valid for other species because a spermatophore is not always present, and other kinds of sperm transfer can exist.

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