# Male genital defect (Dumpton Syndrome) in the dog-whelk *Nucella lapillus* (Neogastropoda): Mendelian inheritance inferred, based on laboratory breeding experiments

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Laboratory breeding of the dog-whelk, *Nucella lapillus*, has established that the male-sterilizing Dumpton Syndrome (DS)—underdevelopment, or non-development (aphally), of the penis, incomplete formation (non-closure) of the vas deferens, resulting in a split prostate—can be readily observed in male F1 progeny. Cultivated under high ambient concentrations of the antifouling agent tributyltin (TBT), DS-carrying females can be recognized by their lesser degree of masculinization (imposex): sterilization is thereby avoided. When Dumpton females are crossed, under high ambient TBT, with individuals from a non-DS-affected population (Bude, North Cornwall) DS is absent from both sexes. Crosses of these F1 progeny result in F2 progeny exhibiting the classic DS symptoms in both sexes. A Mendelian mechanism for DS inheritance is suggested by the data.

## INTRODUCTION

Amongst neogastropods, aberrant development of the reproductive system appears to be extremely rare. Dumpton Syndrome, first discovered at Dumpton Gap, near Broadstairs, north-east Kent (OS grid reference TR 397 666: see Gibbs et al., 1991), is seemingly a unique phenomenon affecting the development of the male genital system of the dog-whelk *Nucella lapillus* (L.) to the extent that the male is incapable of copulation/sperm transfer. During its ontogeny the male gonoduct first appears as an open fold or gutter in the epithelium of the pallial cavity floor running anteriorly from the testis duct to the penis; normally, this gutter sinks below the surface as the edges fuse to create a sunken tube, the vas deferens, to transport sperm across the pallial cavity from the seminal vesicle to the penis (see Gibbs & Bryan, 1994, figure 2). With maturity, the posterior third of the vas deferens becomes swollen and glandular, forming the prostate. A major effect of Dumpton Syndrome (DS), as described in Gibbs (1993a), is the non-fusion of the epithelia forming the 'gutter' edges so that throughout the length of the vas deferens it appears 'split', remaining open to the pallial cavity; posteriorly, a split prostate is a conspicuous feature, the more so because the unfused edges are usually defined by their abnormal brown colour. Importantly, DS also affects penis formation: underdevelopment, or non-development, of the penis is an associated feature. Testis development may also be limited, with spermatozoa formation inhibited. To date the syndrome has not been positively identified in any species other than *N. lapillus*.

Overall, Dumpton Syndrome appears to be highly variable in its degree of severity. In its mildest form the penis exhibits a minor reduction in size and copulation may still be achievable; however, breeding has to be regarded as impossible for males showing a major reduction in penis size, or lacking a penis entirely, and having a split prostate,

coupled, in the extreme state, with an undersized testis lacking the final stages of spermatogenesis. But it is by no means certain that the syndrome would ever have been detected had it not been for the introduction of antifouling paints containing tributyltin (TBT). Following the discovery that exposure to TBT causes females to be masculinized, it was quickly recognized that this phenomenon offered a highly sensitive indicator of TBT pollution (Bryan et al., 1986). Within a few years very many *N. lapillus* samples had been collected throughout the species range in the north-east Atlantic from Portugal to Iceland. Analyses proved imposex to be virtually everywhere, but DS remained undetected until the syndrome was discovered in north-east Kent. The syndrome, or a close variation of it, has subsequently been discovered in the Bay of Brest, north-west France (Huet et al., 1996), and in several Galician rias, north-west Spain (Barreiro et al., 1999; Quintela et al., 2002).

The fact that the syndrome occurs in isolated pockets would seem to indicate that the genital deficiency is the result of a commonly occurring mutation that, until a few decades ago, was maintained at a low level: the frequency of the mutation has now progressed to a readily detectable level because it demasculinizes both sexes; in the male this is catastrophic, but in the female, now almost universally exposed to TBT, the degree of masculinization is lessened: in areas of severe TBT pollution where a normal, non-DS female would be sterilized, such a DS-affected female remains capable of breeding and the population survives.

The masculinizing effect of TBT is known as 'imposex', a term coined by Smith (1971) to describe the superimposition of male organs onto females of gastropods that are normally dioecious species. It is now known that in the case of *N. lapillus* the phenomenon appears on exposure to a concentration of TBT as low as 0.2 ng Sn litre and both penis and vas deferens development become accentuated at higher TBT concentrations. Above a TBT level of 2 ng

Sn litre, male tract tissue invades the space of the female tract so that vas deferens and prostatic elements can be identified within the oviduct (often hyperplasic), causing a blockage that prevents the expulsion of egg capsules and thereby sterilizes the female (see Gibbs & Bryan, 1986; Gibbs et al., 1988). However, in a female carrier of DS it would appear that although a degree of imposex may be apparent, its effects are mitigated, the severest symptoms avoided, with the consequence the female retains her fertility and the population survives despite being in an area where non-DS-afflicted populations have perished. Dumpton Syndrome is thus transmitted, if not solely then mostly, through the female line. One line of evidence suggests TBT may act as a neurotoxin causing release of the peptide hormone penis morphogenic factor (PMF) so as to induce imposex (Oberdörster & McClellan-Green, 2002). However, more work has concentrated on the idea that TBT causes an inhibition of the cytochrome P450-dependent aromatase system responsible for the catalyses of androgen-oestrogen conversion; increased androgen content results in the masculinization of females (Spooner et al., 1991; Bettin et al., 1996; Matthiessen & Gibbs, 1998). Theoretically, DS might limit androgen production, reducing masculinity in males and imposex in females, but the underlying mechanism remains to be established. Laboratory breeding experiments have established that DS is readily apparent in F1 progeny (Gibbs, 1993a). This paper presents further observations of laboratory breeding experiments designed to examine the effects of crossing non-DS-affected males, as well as Dumpton males that appeared normal, with Dumpton females having different degrees of imposex; sexual development in F1 and F2 progenies are then compared.

## MATERIALS AND METHODS

### *Identification of sex and assessment of degrees of imposex and Dumpton Syndrome*

The biology of *Nucella lapillus* is described in Crothers (1985). In common with most neogastropods *N. lapillus* is dioecious. Internal fertilization (copulation) allows the fertilized eggs to be packaged within the oviduct in groups within protective horny capsules (about the size of a wheat-grain) which are expelled and firmly attached to a hard surface, usually intertidal rocks. Larval development takes place entirely within the capsule over a period of three to four months (no planktonic larval phase), after which time the juveniles escape from the capsule as 'crawl-aways', each resembling a miniature adult. In the wild, maturity occurs when about two years old; in sheltered locations individuals may survive for ten years or more. In the laboratory the time taken to reach maturity is often shorter, with females producing capsules at around one year old: probably laboratory conditions such as an abundant food supply (no foraging required), minimal disturbance (no wave action) and a moderated temperature range all combine to accelerate maturation.

Routine determination of sex ratios in large samples of sacrificed de-shelled *N. lapillus* is straightforward because the female is readily recognizable by the dark-coloured sperm-ingesting gland behind the creamy capsule gland. However, in the present study it was necessary for the sex and extent of masculinization to be known and monitored

without damaging the animals over a period of more than two years. This was achieved using magnesium chloride as a narcotic (see Gibbs, 1999), placing the animals in a solution of $MgCl_2.6H_2O$ (75 g in one litre of distilled water) for two hours. In the relaxed state most animals could be eased sufficiently from their shell so as to permit observation of the anterior part of the pallial cavity; thus the penis and much of the vas deferens could be seen. Before the advent of TBT paints these structures were confined to the male and used to identify the sex. But following extensive usage of these paints, females virtually everywhere developed male genitalia and frequently even the moderately affected could be misidentified as being male. However, the female has one secondary sex character seemingly unchanged by TBT exposure: this is the ventral pedal gland, situated on the anterior part of the sole of the foot behind the accessory boring organ (which is found in both sexes). The ventral pedal gland takes the form of a transverse pit to which, during spawning, capsules are passed for final moulding before being pasted to the rock surface. This pit is easily identified on relaxed females if the sole is gently stretched lengthwise. *Nucella lapillus* proved tolerant of such treatment and few failed to recover completely from the operation after being transferred to seawater and left overnight.

Dumpton females were graded according to the degree of their masculinization. The vas deferens sequence (VDS: see Gibbs et al., 1987; Gibbs, 1999) defines six stages (VDS Stages 1–6) but Stage 1 can only be determined if the genital papilla area in the posterior part of the pallial cavity can be examined; this is not possible without shell removal followed by a longitudinal incision of the pallial cavity roof, necessitating a sacrifice of the female. Similarly, Stages 4, 5 and 6 cannot be separated and have to be lumped together. Under narcosis, females appearing to be normal and females exhibiting three stages or types of imposex were distinguished: (i) type 0, no sign of imposex (VDS Stage 0, but could be Stage 1); (ii) type np, no penis but vas deferens developed (aphallic VDS Stages 4, 5 or 6); (iii) type sp, a short penis (<2 mm in length) and with a vas deferens ('normal' VDS Stages 4, 5 or 6); (iv) type lp, a long penis (≥2 mm in length) and with a vas deferens ('normal' VDS Stages 4, 5 or 6).

In the males, the extent of DS varied; some had a full-sized penis and were seemingly unaffected ('normal'); others either had an under-sized penis or lacked a penis entirely, but the vas deferens appeared intact (DS type 1); in the worst affected (DS type 2) the vas deferens appeared unfused along much, or all, of its length, most noticeably in the posterior prostate section ('split' condition); penis formation was variable in extent but usually totally absent. All of DS type 2 and probably many of DS type 1 were regarded as infertile insofar as sperm transfer seemed impossible. These males were discarded, and only those classified as normal were selected as breeders.

### *Experimental*

The shells of females collected from Dumpton Gap in March 1990 were marked with waterproof ink for identification and all were kept isolated from males for a period of six months to allow any stored sperm from any earlier field matings to be eliminated. In October 1990 the

females were again narcotized to confirm the degree of masculinization of each was the same as in March. Ten females of each of the four imposex types ((i)–(iv)) described above were selected. Each of these four groups was then divided into two and the resulting two sets of four (i.e. eight batches in total) were all placed in separate containers (closed plastic freezer baskets). Twenty Dumpton males with normal-sized penises were then selected and five placed in each of one of the sets of four. This procedure was repeated for the other set but in these baskets recently-collected males from Bude (see below) were used. All eight containers were then placed in tidal tanks supplied with laboratory circulation water. The tributyltin level in the water supply was enhanced by passing it through a chamber in which a rod coated with TBT copolymer paint was immersed. Tributyltin concentrations in the tank water (which could be adjusted by raising or lowering the rod) were determined at intervals of two to three weeks (see Bryan et al., 1986 for method). Over the 30-month period January 1991 to June 1993 the mean concentration was 3.8 ng Sn litre (SD 2.04; range 1.3–9.4; N=41). From the results of earlier experiments (Gibbs et al., 1988), it was known that maintaining the TBT concentration within this range would cause imposex to be expressed to its full extent in *N. lapillus* progeny by the time they had reached maturity at an age of between one and two years. This proved the case in this experiment.

*Nucella lapillus* at Bude, North Cornwall (OS grid reference SS 201 072) had been monitored every six to eight weeks between 1985 and 1991: during this period several thousand males were examined but none showed any abnormality of the genitalia, such as found at Dumpton Gap. The north Cornish population can be considered typical of the species overall (illustrated in Gibbs, 1993b) and suitable for investigating the nature of inheritance of Dumpton Syndrome, given that Bude and Dumpton Gap are separated by over 800 km of coastline and the life cycle of *N. lapillus* does not include a dispersive planktonic phase. (The two populations have been isolated since the Channel was formed during the post-glacial period, some 10,000 ya.) However, it is well-known that *N. lapillus* populations vary in their chromosome complements, exhibiting Robertsonian polymorphism (2n=26–36: see Dixon et al.,

1994) and a degree of incompatibility between individuals with widely differing complements is possible. Pascoe (2002) failed to obtain viable offspring from attempted crosses of three chromosomally-different populations but the fact that the two populations used here have a similar number, i.e. Bude has 2n=26, and Dumpton 2n=26–28 (Pascoe, 2002) may have contributed towards obtaining a successful outcome.

The capsules resulting from each of the batches or mating groups were kept separately in small-mesh cages and the resulting young (F1) reared on small mussels and barnacles until 10–12 months old. At this stage the onset of maturity was approaching (no capsules had yet been produced) and individuals in each group were examined under narcosis to determine sex and the levels of DS and imposex. Selected F1 females were then isolated from males to prevent copulation; no capsules were produced over the ensuing six-week period so it is assumed no copulations had occurred prior to isolation and therefore no stored sperm could be present. Ten of these females were caged with ten normal F1 males; when capsule production ceased after several months, these F1 animals were returned to their original cages for the later assessment of all F1 animals when aged 22–24 months. Capsules resulting from the second mating were isolated and the young (F2) cultured to maturity at an age of around 12 months when assessments of DS and imposex were possible. The schedule is summarized in Table 1.

## OBSERVATIONS AND RESULTS

The details of the four batches of Dumpton females selected from the March 1990 field sample are given in Table 2. Pre-experimental narcosis proved successful as a method of distinguishing the four states of masculinization in that all details were confirmed when the females were finally sacrifced after seven months; for example, no female put in the 0 or np categories, was subsequently found to possess a penis. As expected, those females with a short penis were subsequently found to be still capable of laying capsules (VDS Stage 4) whilst those carrying a long penis were generally sterilized (VDS Stages 5 or 6). Overall survival was good: of the 40 Dumpton females

**Table 1.** *Schedule of stages in breeding crosses between Dumpton males (normal), and Bude males, with Dumpton females (types D0, Dnp, Dsp, and Dlp: details in text).*

| Year | J | F | M | A | M | J | J | A | S | O | N | D |
|------|---|---|---|---|---|---|---|---|---|---|---|---|
| 1990 |   |   | 1 |   |   |   |   |   |   | 2 |   |   |
| 1991 | 3 | 3 |   | 4 |   |   |   |   |   |   | 5 | 6 |
| 1992 |   |   |   | 7 | 7 |   |   |   |   |   | 8 |   |
| 1993 |   |   |   |   |   | 9 |   |   |   |   |   |   |

1. Sample collected at Dumpton Gap, assessed under narcosis and sexes separated.
2. Sample collected at Bude. Sexed under narcosis. Bude males and Dumpton type 0 males combined with Dumpton female types D0, Dnp, Dsp and Dlp. Capsules produced (Table 3).
3. Capsules from each batch transferred to isolated small-mesh cages.
4. F0 animals de-shelled and fully assessed (Table 2).
5. F1 progeny assessed under narcosis (Table 4). Potential parents of F2 generation selected and isolated.
6. Parents of F2 brought together. Capsules produced.
7. Capsules from each batch transferred to isolated small-mesh cages.
8. F1 animals, 22–24 months old, de-shelled and fully assessed (Tables 5 & 6).
9. F2 animals, 12–14 months old, de-shelled and fully assessed (Tables 5 & 6).

**Table 2**. *Pre- (October 1990) and post-experimental (April 1991) assessments of masculinization of Dumpton females, Dumpton males and Bude males (five females and five males in each batch) used as parents of Dumpton×Dumpton and Dumpton×Bude F1 generations. See text for details of pre-experimental batch codings under narcotization (left-hand columns). Some individuals died (marked d) and one found heavily infected by trematode parasite(p).*

| Batch code | Pre-experiment assessment | Post-experiment degree of masculinization of F1 parents used in breeding crosses | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dumpton females with Dumpton males | | | | | Dumpton females with Bude males | | | | |
| | | Dumpton females—VDS Stage | | | | | Dumpton females—VDS Stage | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| np | 4–6np | 4np | 4np | 4np | 4np | d | 4np | 4np | 4np | 4np | 4np |
| sp | 4–6sp | 4 | 4 | 4 | 4 | d | 4 | 4 | 4 | 4 | d |
| lp | 4–6lp | d | 5 | 6 | 6 | 4/5 | 4/5 | 6 | 5 | 6 | 6 |
| | | Dumpton females—penis length (mm) | | | | | Dumpton females—penis length (mm) | | | | |
| 0 | 0 | — | — | — | — | — | — | — | — | — | — |
| np | 4–6np | — | — | — | — | — | — | — | — | — | — |
| sp | 4–6sp | 1.7 | 2.1 | 1.6 | 1.5 | d | 1.7 | 1.5 | 1.3 | 1.6 | d |
| lp | 4–6lp | d | 3.2 | 4.0 | 3.0 | 3.2 | 3.1 | 3.1 | 3.8 | 3.9 | 3.7 |
| | | Dumpton males—penis length (mm) | | | | | Bude males—penis length (mm) | | | | |
| 0 | | 4.5 | 4.3 | 3.7 | 4.7 | d | 3.5 | 4.2 | 4.3 | 4.0 | 4.2 |
| np | | 4.2 | 4.5 | 3.8 | 4.4 | 4.6 | 3.3 | 4.0 | 3.9 | 4.3 | 3.8 |
| sp | | 4.0 | 3.4 | 4.2 | p | 4.7 | 4.5 | 4.5 | 4.0 | 4.3 | 4.4 |
| lp | | 3.7 | 3.6 | 3.4 | 4.2 | 3.9 | 4.1 | 4.0 | 4.6 | 4.3 | d |

used, just four died within the six months when breeding took place, all having been in captivity for over a year.

The capsule productions of the eight batches are given in Table 3. The mean numbers of capsules laid per female in the 0, np and sp batches are comparable. Predictably, females in both lp batches generally proved to be infertile through oviduct blockage, but both batches had one female seemingly nearly but not actually sterilized (VDS Stages 4/5). These females were, most likely, responsible for the few capsules laid in the lp batches; however, these particular capsules were mostly smaller than average, some empty and the remainder contained irregular yolk masses. No hatchlings were recovered.

When six–eight months old, all six Fl batches each comprised 200–300 surviving juveniles. When these became sub-adult (close to one year old) they were examined under narcosis (Table 4).

The aims were to identify the sexes, any individual exhibiting aphally, and to select possible parents for producing an F2 generation, whilst keeping the animals alive to allow further sexual maturation. Since the TBT concentration in the ambient seawater was maintained at a level above that inducing full imposex ($>2$ ng Sn litre), it can be predicted those males and females unaffected by DS will all possess a penis, whereas either sex with DS will be aphallic. The difference between the two sets of progeny is striking: whereas all three batches mating Dumpton animals produced aphallic individuals of both sexes (twice as many females as males), those males and 276 females obtained from three Bude/Dumpton batches were all phallic.

The condition of the Fl males surviving to sexual maturity (in this case at 22–24 months old), are summarized in Table 5. Fully developed DS (split prostate, type 2) appeared in all three 'pure' Dumpton batches (6–25%). Penis lengths show wide and similar distribution (Figure 1). In samples of 34 or 35 taken from the stocks of the Bude × Dumpton crosses, no male showed any sympton of DS, confirming the results of the examination earlier under narcosis; penis lengths are comparable in distribution in the three batches (Figure 1).

The condition of the Fl females surviving to maturity are summarized in Table 6. The effect of the ambient TBT is seen in the status of imposex, which, in all cases, reached VDS Stage 4 and above. Importantly, none of the sterilized females (VDS Stages 5 and 6) was aphallic, indicating none was a DS-carrier and was thus liable to suffer the full effects of TBT exposure. Females at VDS Stage 4 and aphallic probably represent the DS-carrying element; these were present in each of the three batches, constituting 8–22% of the totals. Penis lengths exhibited a wide, but similar, variation (Figure 2). In contrast, in

**Table 3.** *Number of egg capsules produced (batch total, mean per female) by Dumpton females exhibiting varying masculinization (types 0, np, sp, lp: see text) when mated with either Dumpton males or Bude males.*

| Dumpton female | ×Dumpton male | | ×Bude male | |
|---|---|---|---|---|
| | Batch total | Mean/female | Batch total | Mean/female |
| 0 type | 249 | 50 | 291 | 58 |
| np type | 234 | 47 | 186 | 37 |
| sp type | 238 | 48 | 151 | 30 |
| lp type | 1 | — | 39 | 8 |

**Table 4.** *Penis development at age 10–12 months (November 1991) in F1 offspring of crosses between Dumpton females exhibiting different degrees of masculinity (column 2) and Dumpton/Bude males (column 1). D0, Dnp and Dsp—see text. Indet = indeterminable individual not included in census because either (a) pedal gland present but rudimentary, or (b) specimen not sufficiently protruded from shell.*

| Parents | | Offspring (numbers) | | | | | |
|---|---|---|---|---|---|---|---|
| Males origin | Females imposex state | Males | | Females | | Indet | |
| | | Penis | No penis | Penis | No penis | a | b |
| Dumpton | Dumpton [0] VDS 0 | 89 | 2 | 59 | 11 | 5 | 3 |
| Dumpton | Dumpton [np] VDS 4np | 98 | 4 | 67 | 4 | 6 | 0 |
| Dumpton | Dumpton [sp] VDS 4 | 103 | 12 | 95 | 20 | 0 | 1 |
| Bude | Dumpton [0] VDS 0 | 101 | 0 | 74 | 0 | 6 | 15 |
| Bude | Dumpton [np] VDS 4np | 100 | 0 | 92 | 0 | 1 | 1 |
| Bude | Dumpton [sp] VDS 4 | 125 | 0 | 110 | 0 | 14 | 3 |
| Totals | | | | | | | |
| Dumpton | Dumpton | | | | | | |
| ($n=564$) | [% aphallic] | 290 | 18 [5.8] | 221 | 35 [13.7] | | |
| Bude | Dumpton | | | | | | |
| ($n=602$) | | 326 | 0 | 276 | 0 | | |

samples of 35 to 37 taken from each of the three Bude × Dumpton stocks aphally was absent (as when these individuals were examined under narcosis earlier in their life (Table 4)). The majority were sterilized by imposex: in all cases the penis was well-developed (Figure 2); in fact, the profile of the length distributions for the female penis closely follow those for the corresponding male penis; recognition of the sexes on the basis of penis length was thus impossible.

In choosing the parents for producing the F2 generation, ten F1 males were selected from the progeny of the Bude males×Dumpton females without imposex (VDS Stage 0) as this cross was regarded as offering the best possibility of handing down DS syndrome. The females needed to be isolated sometime before becoming sexually active in order to avoid inappropriate impregnation; ten of the 20 F1 aphallic females that could be reliably recognized as such before reaching one year old (i.e. before
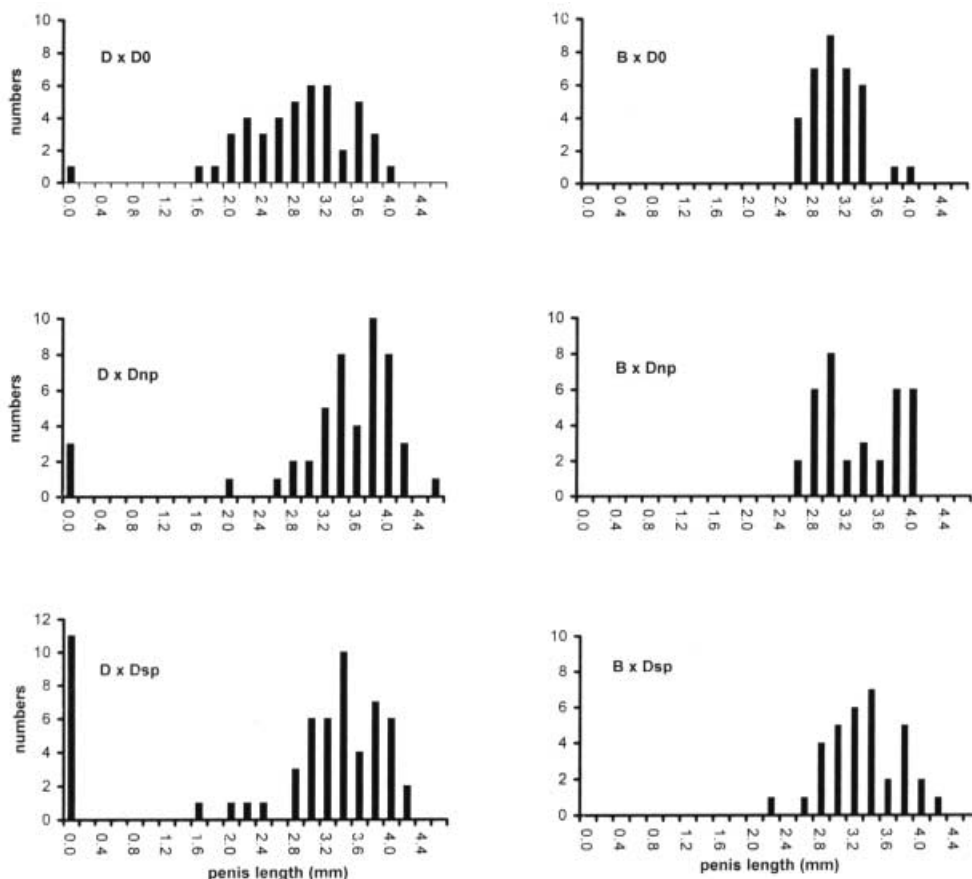


**Figure 1.** *Nucella lapillus* males. Frequency distributions of penis length in F1 populations examined at 22–24 months old. Sample numbers and abbreviations are as given in Table 5.
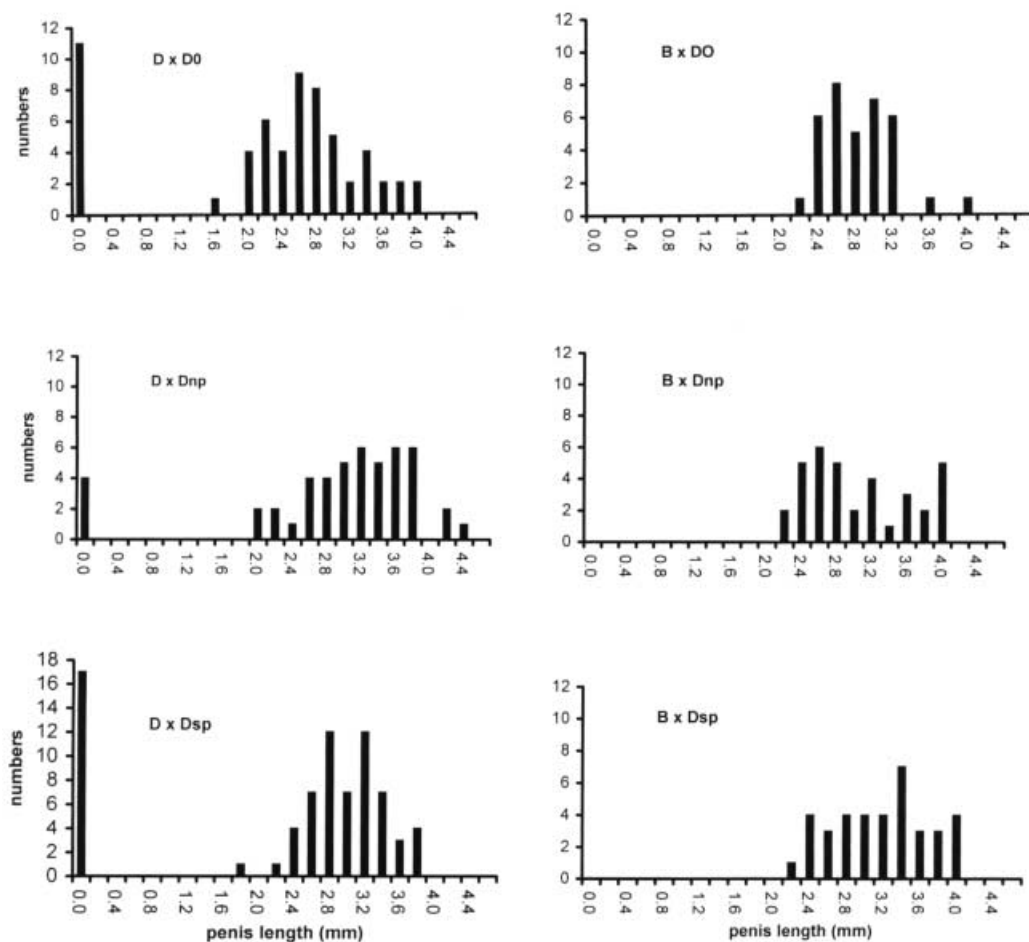
**Figure 2.** *Nucella lapillus* females. Frequency distributions of penis length in F1 populations examined at 22–24 months old. Sample numbers and abbreviations are as given in Table 6.

maturity) in the D×Dsp cross (Table 4) were placed with ten males from the B×D0 mating. No female of this group had produced a capsule during isolation.

When *Nucella lapillus* is kept in captivity over a long period of time, it is not unusual to find the occasional individual that has shed its shell and it may survive several weeks in this 'naked' state before dying. The operculum may also be shed. (The cause of such autonomous 'de-shelling' is not known but inactivity may be contributory: Crothers (1977) thought such mortality might be the result of being raised in still water.) During the rearing of the F2 generation, mortality through such de-shelling became increasingly common and a complete loss of the progeny seemed possible. Thus it was decided to carry out

**Table 5.** Nucella lapillus *males. Expression of Dumpton Syndrome in F1 and F2 laboratory-bred populations. F1 was examined when 22–24 months old, F2 when 12–14 months old.*

| Cross m×f | Sample number | 'normal' | Prostate condition 'split' | | DS-affected (type 2) | |
|---|---|---|---|---|---|---|
| | | | penis | no penis | *n* | % |
| F1 population | | | | | | |
| D×D0 | 45 | 41 | 3 | 1 | 4 | 8.9 |
| D×Dnp | 48 | 45 | 0 | 3 | 3 | 6.3 |
| D×Dsp | 60 | 45 | 4 | 11 | 15 | 25.0 |
| Total | 153 | 131 | 7 | 15 | 22 | 14.4 |
| | | | | | | |
| B×D0 | 35 | **35*** | 0 | 0 | 0 | 0 |
| B×Dnp | 35 | 35 | 0 | 0 | 0 | 0 |
| B×Dsp | 34 | 34 | 0 | 0 | 0 | 0 |
| Total | 104 | 104 | 0 | 0 | 0 | 0 |
| | | | | | | |
| F2 population | | | | | | |
| [B×D0]×[D×Dsp]np | 40 | 27 | 11 | 2 | 13 | 32.5 |

Males (m): D=Dumpton, B=Bude. Females (f): D0, Dnp, Dsp—see text. F2 derived from cross of [B×D0] males (asterisked) and aphallic [D×Dsp] females (see Table 6).

**Table 6.** Nucella lapillus *females. Expression of Dumpton Syndrome in F1 and F2 laboratory-bred populations. F1 was examined when 22–24 months old, F2 when 12–14 months old.*

| Cross m×f | Sample number | 0 | 1 | 2 | 3 p | 3 np | 4 p | 4 np | 5 p | 5 np | 6 p | 6 np | DS-affected (aphallic) n | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **F1 population** | | | | | | | | | | | | | | |
| D×D0 | 60 | 0 | 0 | 0 | 0 | 0 | 39 | 11 | 9 | 0 | 1 | 0 | 11 | 18.3 |
| D×Dnp | 48 | 0 | 0 | 0 | 0 | 0 | 29 | 4 | 13 | 0 | 2 | 0 | 4 | 8.3 |
| D×Dsp | 77 | 0 | 0 | 0 | 0 | 0 | 31 | 17* | 26 | 0 | 3 | 0 | 17 | 22.1 |
| Total | 185 | 0 | 0 | 0 | 0 | 0 | 99 | 32 | 48 | 0 | 6 | 0 | 32 | 17.3 |
| | | | | | | | | | | | | | | |
| B×D0 | 35 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 19 | 0 | 0 | 0 | 0 | 0 |
| B×Dnp | 35 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 16 | 0 | 0 | 0 | 0 | 0 |
| B×Dsp | 37 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 22 | 0 | 3 | 0 | 0 | 0 |
| Total | 104 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 57 | 0 | 3 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | |
| **F2 population** [B×D0] [D×Dsp]np | 37 | 0 | 0 | 0 | 0 | 0 | 29 | 8 | † | † | 0 | 0 | 8 | 21.6 |

Males (m): D=Dumpton, B=Bude. Females (f): D0, Dnp, Dsp—see text. VDS Stages: p, with penis: np, no penis. F2 derived from cross of [B×D0] males (see Table 5) and aphallic (np) [D×Dsp] females (asterisked). †Note that VDS Stages 4 and 5 are indistinguishable when females are immature, as in F2 generation.

the final assessment earlier than planned; the brood was just 12–14 months old when examined but nevertheless was approaching maturity. Importantly, the characteristic abnormality of DS—a split prostate—absent in the F1 generation, reappeared in about a third of the F2 male survivors (Table 5). Of the F2 female survivors, about a fifth proved to be aphallic (Table 6).

## DISCUSSION

Dumpton Syndrome can be interpreted as a continuum rather than an all-or-nothing phenomenon. It manifests itself as a variable underdevelopment of the male genital tract. Affected males may show a minor reduction of penis size, a total absence of penis, a reduced vas deferens, or partial-to-complete non-closure of the pallial sperm duct to give the classic symptom of the split prostate. The sperm duct of the most affected recalls the condition of more primitive gastropods in which sperm are transported across the pallial cavity in an open gutter or groove rather than the evolved sunken tube as is found in the more advanced neogastropods (Fretter & Graham, 1962). In this respect one could regard DS as a reversal, or arresting, of ontogeny recapitulating phylogeny. The cause of the syndrome is unknown but since studies have shown testosterone causes females to become masculinized it seems likely DS is a reversal of this effect, i.e. DS-affected males have insufficient testosterone to effect the normal development of the male genitalia. Likewise the DS-affected female reacts in a variable manner to TBT exposure; in some, imposex develops but not fully, in others it is seemingly absent altogether. This could be because they are incapable of producing the required excess of testosterone when exposed to TBT. Steroid metabolism has been studied in *Nucella lapillus* (Spooner et al., 1991; Bettin et al., 1996); however, the steroid titres of variously DS-affected individuals of both sexes from the Dumpton population have yet to be determined.

During copulation sperm are deposited in the bursa copulatrix within the capsule gland complex of the oviduct. This is situated at the base of the pallial cavity and to access the vulva the penis must be not only highly extensible but also of a minimal length. Looking at the penis length data for the Dumpton population it is obvious that the penis of many males is far too short to effect fertilization. Deciding what is the minimal length is not straightforward but if we assume all males in a typical population such as at Renney Rocks, Plymouth Sound, are capable of fertilization then the minimum length of around 3.4 mm emerges (see Gibbs, 1993a figure 5C). In the laboratory-bred F1 Dumpton males, penis length was below this 3.4 mm minimum (including complete absences) in 76%, 29% and 51% in the D×D0, D×Dnp and D×Dsp batches respectively; comparable percentages for Bude-Dumpton-cross males were 77, 51 and 50, but wholly without aphally. Thus, in round terms, about 50% of males appeared physically incapable of successful copulation. Each of the frequency histograms for F1 male penis length in all six crosses (Figure 1) has an indication of bimodality; possibly one component group may differ genetically from the other. However, sample sizes are too small to permit a computer-based analyses (see Grant, 1989) to refine this interpretation. Penis lengths in F1 females of both crosses show similar size distributions but lack any modality. Dumpton Syndrome has a subtle effect on the process of masculinization whether natural, as during the ontogeny of males, or artificially imposed on females, as imposex through exposure to a pollutant such as organotin.

From the data it is clear that DS is a recessive trait since the F1 progeny of the Bude male-Dumpton female crosses never expressed DS. Also DS is highly variable in its expression in both sexes and in selecting the parents for the breeding crosses only one anatomical feature was available to judge its character in the living animal, notably the extent of penis development. The size of the female penis relative to the size of the male penis for any

given population can be used as an indicator of the severity of exposure to TBT: the relative penis size index (RPSI) (Gibbs et al., 1987) has been widely employed for *N. lapillus* imposex surveys. Since DS reduces masculinization to a variable degree in males, and also in TBT-exposed females, for experimental purposes the size of the penis (in narcotized animals of both sexes) was used to estimate the degree of DS imposition. If this assumption is correct then the selected Dumpton males with full-size penises, potentially capable of copulation (Table 2), should be normal, similar to Bude males and not carry DS (i.e. be homozygous NN); when mated with females fully DS-afflicted (recessive homozygous, dd) without imposex (VDS Stage 0) then no progeny with DS should appear if Mendelian rules apply. However, DS-afflicted F1 progeny were produced (Tables 5 & 6) and it has to be concluded that one, some, or all the male parents were heterozygote, (Nd). Thus penis development cannot be considered a good indicator of DS severity, in which case the modal distributions of male penis length (Figure 1) mentioned above probably have no direct relation to genetic make-up.

As with the aphally of imposexed females, the key character of fully developed DS—a split prostate—did not appear in the F1 generation of the Dumpton×Bude cross but reappeared in the F2 generation. The ratio of normal to DS-affected in F2 males was roughly 2:1 (27:13); for females this ratio was somewhat less than 4:1 (29:8). A Mendelian mechanism for the inheritance of DS would appear likely but, because of high mortality within the experimental batches, sample numbers are too low to be conclusive in respect of ratios.

Results can be summarized thus: Dumpton Syndrome is caused by a recessive allele, possibly monogenic, that reveals its presence in *N. lapillus* populations subject to high TBT pollution through causing a deficiency in the ontogeny of the male genital tract. DS-afflicted males are impotent. Non-DS-afflicted females are infertile because of sterilization by TBT-induced imposex. Only DS-afflicted females are fertile under these conditions and these can breed with non-DS-afflicted males.

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