Evolutionary trends in feminization and intersexuality in woodlice (Crustacea, Isopoda) infected with *Wolbachia pipientis* (*α-Proteobacteria*)

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ABSTRACT. Sex ratio distortion (SRD) and intersexuality are common phenomena among Isopoda (Arthropoda, Crustacea), caused by the feminizing action of the α-Proteobacterium *Wolbachia* (F) and/or an F DNA segment (f) integrated into the host genome, probably as a transposon. A dominant autosomal masculinizing allele (M) overrides the primary sex determinants (WZ: females; ZZ: males) and f but not F. The latter can be counterbalanced by the transmission suppressor polygenic system (R). The present study pursued a double object. (i) SRD was found in naturally isolated populations of three out of seven Belgian woodlouse species: *Oniscus asellus*, *Armadillidium vulgare* and *A. pulchellum*. They were (inter)sexed based on external and internal morphology, and specimens of the former two underwent PCR and/or microscopic detection of F. (ii) A deterministic but flexible model was set up to describe SRD changes in an initially F-infested *A. vulgare* population sampled during the last 25 years. Observed and predicted sex ratios fit best if natural transmission rates (i.e., in the absence of M and R) approach 100%, a condition fulfilled if f is carried on a multicopy transposon. Most often, such a population will then gradually lose its invader to the benefit of f. The stable end situation is a host population consisting of ZZ+f individuals of which 44% are neo-females (having mm), and 56% are reversed males (owing to Mm or MM). The model also explains the accelerated SRD evolution under variable degrees of F/f transmission.

KEY WORDS : Isopoda, feminization, intersexuality, sex ratio evolution, *Wolbachia*.

INTRODUCTION

(Abbreviations used: IR, intersex ratio; SR, sex ratio; SRD, sex ratio distortion)

Isopoda (Crustacea, Malacostraca) or woodlice, along with their relatives the Amphipoda, are the only crustaceans of which strictly terrestrial species exist. About 4000 out of a total of 8000 isopod species described are terrestrial; 33 of these belong to the Belgian fauna, which was the starting point of this study.

Sex seems to be determined rather uniformly on a hormonal basis throughout the Crustacea (LEGRAND et al., 1987). However, more than 40% of the terrestrial woodlouse species have been reported to display sex ratio distortion (SRD) towards the female sex (JUCHAULT & LEGRAND, 1989; JUCHAULT et al., 1994; BOUCHON et al., 1998) whereas most aquatic counterparts have a sex ratio (SR) close to 1:1. SRD and intersexuality in isopods have been ascribed to the feminizing action of an obligately-intracellular and maternally-inherited bacterium originally denoted F (MARTIN et al., 1973) but later determined as the α-*Proteobacteria* member *Wolbachia pipientis* (ROUSSET et al., 1992), and also to a wolbachial DNA fragment: f (JUCHAULT et al., 1992). The latter may be unstably integrated into the host genome, maybe as a transposable element; therefore, unlike F it can be partly paternally inherited (JUCHAULT & MOCQUARD, 1993) and so introgress into populations harbouring F (GRANDJEAN et al., 1993).

F can be horizontally transmitted by transplanting infected organs (LEGRAND & JUCHAULT, 1970) or by inoculation (JUCHAULT & MOCQUARD, 1989), whereas f cannot spread horizontally (LEGRAND & JUCHAULT, 1984). Neither can it be eliminated, unlike F, by antibiotics or temporarily elevated temperatures (JUCHAULT et al., 1980b; RIGAUD et al., 1991a, 1997; RIGAUD & JUCHAULT, 1998). A strikingly similar temperature-dependent SRD effect in Amphipoda results from the action of parasitic Microsporidia (GINSBURGER-VOGEL & CARRÉ-LÉCUYER, 1976; BULNHEIM, 1978; GINSBURGER-VOGEL et al., 1980; GINSBURGER-VOGEL, 1991).

The longest and best documented Isopoda case is *Armadillidium vulgare* (LEGRAND & JUCHAULT, 1969; MARTIN et al., 1973; JUCHAULT & LEGRAND, 1976, 1981a,b; JUCHAULT et al., 1980a; RIGAUD et al., 1991b). Here, the female:male ratio in an F-infested population frequently exceeds 10:1. Genetic females are heterogametic WZ, males are homogametic ZZ (JUCHAULT & LEGRAND, 1972). Transformation into so-called neo-females usually takes place in an early embryonic stage: the parasitic sex

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factors inhibit the expression of male genes carried by the Z heterochromosome, thereby preventing the growth of the androgenous gland, which would induce the development of the male gonads (SUZUKI & YAMASAKI, 1991, 1997) through secretion of androgenic hormone (MARTIN et al., 1999).

In some populations SRD is less dramatic. This may result from three possible factors. Firstly, the onset of F expression may occur later in ontogeny, giving rise to incompletely feminized intersexes of one of two categories (MARTIN et al., 1973; LEGRAND et al., 1974): iF having a functional female phenotype with vestigial gonopods, and iM being sterile male-like individuals with reduced gonopods and gonads. There is growing evidence that the dominant allele of an autosomal masculinizing gene (M) slows down *Wolbachia* proliferation (RIGAUD & JUCHAULT, 1993), hence the postponed impact of F. Sometimes this may even happen in the absence of M, albeit in a much less pronounced manner. Secondly, f as well as the primary W female sex determinant, is overruled by M, thus masculinizing even genotypic females provided they are not infected by F (RIGAUD & JUCHAULT, 1993). Moreover, the genetic constitution WZMm may lead to yet another intersex type if the effect of M is delayed: a functional male possessing also two female genital orifices (denoted M_{og} throughout this paper) (LEGRAND et al., 1974; JUCHAULT & LEGRAND, 1976). Furthermore, it is especially M that allows a high paternal transmission rate of f (JUCHAULT et al., 1992). Thirdly, a polygenic "Resistance" (R) system limits the transovarial transmission of *Wolbachia* to offspring. Such infected mothers no longer produce a female-skewed F_1; on the contrary, a few of them are overwhelmingly male-biased, a phenomenon referred to as "ARF (ARrhenogenous Female) trait" (LEGRAND & JUCHAULT, 1972; RIGAUD & JUCHAULT, 1992).

Only in wild infected populations can M, and presumably also R mechanisms, be observed (RIGAUD & JUCHAULT, 1993). Their selection is a naturally genetic response counteracting the selfish wolbachial genes in the struggle for maximal transmission. As a result, males are saved from extinction. An overview of the complex and concurrent sex determination systems in *Wolbachia*-infected woodlice is shown in Fig. 1; the R genes are omitted since these exert only indirect influence on sex development.

The major aim of this work is to examine how far the M and R systems support recovery of the male sex in nature. To achieve this, we resorted to two complementary approaches. (i) We screened populations of seven common Belgian woodlouse species in the suborder Oniscidea for SRD and infections, and compared our results for Belgium with those obtained by BOUCHON et al. (1998) mainly for France. (ii) In the wake of RIGAUD et al. (1992), JUCHAULT et al. (1993), and CAUBET et al. (2000), we present a new mathematical model for the SR changes in *A. vulgare* populations starting from infestation by F. It takes account of

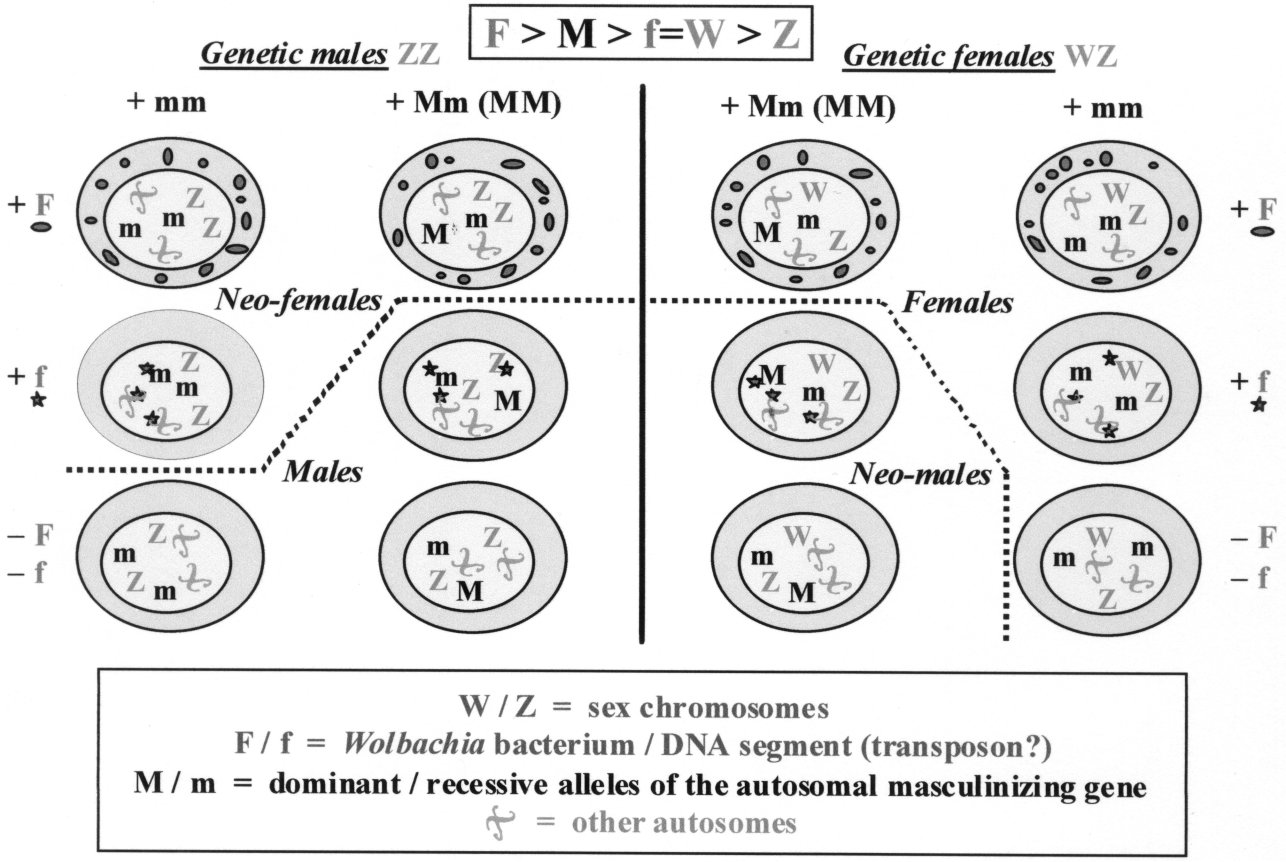

Fig. 1. – Scheme of the concurrent sex determination systems in *A. vulgare*. Note that only three out of six classes of phenotypic males are retained on the whole (but no more than one out of four in the presence of wolbachial factors), whereas only two (one, respectively) classes of genotypic females can be transformed to phenotypic neo-males if M is present. The hierarchical (= epistatic) order of influence of the different factors is also shown.

the M and R genes in addition to f and F, and it is discussed in the context of our observations in nature.

MATERIAL AND METHODS

Isopods

The isopod species were determined using the key of HOPKIN (1991). Populations investigated are displayed in Table 1, along with total numbers of manually-collected individuals, the date of collection (including old samples in addition to recent ones) and their SR. The limit for SRD was arbitrarily drawn at 40% males. The specimens caught before 1995 were fixed and preserved in 70% ethanol with added glycerine, whereas those from 1995 onwards were kept alive upon capture until further specific treatment (see below). They were (inter)sexed by the shape of the endopodites of the first two pleopod pairs, which grow into copulatory organs in males, and/or by internal genital morphology.

Consistently hand-collected samples avoided all noise variation in recorded SR but that originating from the time of the year the isopods were caught. Males are the more active, more exposed sex during the reproductive period (DANGERFIELD & HASSALL, 1994), the start and duration of which depend largely on the latitude with its characteristic photoperiod (for Belgium: May to September) (SOUTY-GROSSET et al., 1994). Thus, especially the observations from May might be an overestimate, and those from autumn or winter a slight underestimate of the real SR. In order to avoid this source of background noise as much as possible, animals were dug out from various depths of sheltered areas and additionally, we carefully rummaged through the soil surface for active individuals.

TABLE1

Isopod species, populations, sampling dates, total numbers of adult individuals and SR (expressed as % males). SRD cases (< 40% males) are typed in boldface.

Species	Populations	Date	#	SR (%)
Armadillidium opacum (Armadillidiidae) (Koch, 1841)	Sint-Genesius-Rode: Zoniënwoud	09/1997	54	51.9 (-)
A. pulchellum (Armadillidiidae) (Zencker, 1798)	Lanaken: Gellikerheide	08/1997	200	**28.5**
		09/1997	73	**28.8**
A. vulgare (Armadillidiidae) (Latreille, 1802)	Nieuwpoort: military domain	05/1977	136	**22.0**
		05/1979	308	**16.9**
		05/1980	132	**25.0**
		10/1980	169	**23.7**
		01/1981	145	**30.3**
		11/1995	100	**34.0** (F)
		11/1997	194	41.8 (F)
	Gent: university, botanical garden	11/1995	196	54.1 (-)
		11/1997	136	48.5 (-)
Ligidium hypnorum (Ligiidae) (Cuvier, 1792)	Waasmunster: spring forest	05/2000	34	41.2 (-)
Oniscus asellus (Oniscidae) Linnaeus, 1758	Waasmunster: spring forest	05/2000	96	**22.9** (F)
	Gent: Bourgoyen nature reserve	05/2000	16	43.8 (-)
Philoscia muscorum (Oniscidae) (Scopoli, 1763)	Nieuwpoort: military domain	03/1980	540	46.9
		10/1980	348	60.9
	Waasmunster: spring forest	05/2000	70	40.0 (-)
Porcellio scaber (Porcellionidae) Latreille, 1804	Nieuwpoort: military domain	03/1980	203	46.3
		10/1980	275	51.6
	Waasmunster: spring forest	05/2000	96	44.8 (-)

(F) Feminizing *Wolbachia* detected by means of DAPI fluorescence microscopy and/or PCR
(-) No F-like bacteria found either by DAPI or PCR; see Table 2 for examples of more detailed (inter)sex analysis

PCR detection of *Wolbachia*

A. vulgare wolbachial DNA was detected as follows. All glassware was sterilized before use. The animals were anaesthetized and surface-sterilized through a three-step 96% ethanol bath (5 min each, followed by rinses in a sterile 0.85% [w/v] NaCl solution), and fixed 30 min in freshly made 50% (v/v) acetic acid. While animals were submerged in the final rinsing solution, the bilateral V-shaped fat bodies and the reproductive organs were dissected out with fine glass needles. Care was taken not to puncture the gut containing a contaminant flora. Whole DNA from the separate organs was prepared and intracellular bacterial 16S rRNA genes PCR-amplified. To determine the presence of *Wolbachia*, partial sequencing of the 16S rDNA was done using two conserved primers: one forward (*Escherichia coli* positions 339-358) and one reverse (*E. coli* positions 536-519). For details on molecular methods, see VANDEKERCKHOVE et al. (2000).

Fluorescence microscopy

The woodlice were anaesthetized, surface-sterilized, fixed, rinsed and dissected as described above. For details on DAPI (4',6-diamidino-2-phenylindole) fluorescence microscopy, see COOMANS et al. (2000) and VANDEKERCKHOVE et al. (2002).

Detection by the DAPI method proved to outperform conventional PCR trials; therefore, most animals were examined using DAPI alone. Although it is not a specific stain, *Wolbachia*-like bacteria could be routinely distinguished by their morphological characteristics (cells are often extremely small, not more than 300 nm, with a marked pleomorphy) once 16S rDNA sequence analysis had unambiguously confirmed the identity of *Wolbachia pipientis* in the organs concerned.

Evolutionary ecological modelling

A simple deterministic model was set up to describe short-term SRD evolution in *A. vulgare*, starting from an F-infested population such as the one from Nieuwpoort in 1977. It must be pointed out that the evolution from the uninfected state (SR 1:1) over F invasion and subsequent sweep to heavy SRD is not applicable here because this part of the micro-evolutionary SR curve obeys quite different dynamics. To test the model on the male recovery side, its mathematical function was cast into a Turbo Pascal program. A whole gamut of parameter values was entered, including figure combinations under several realistic circumstances, and a computer-simulated course of evolution yielded an outcome of the model that could be compared to what was observed in the field.

RESULTS AND DISCUSSION

Prevalence of *Wolbachia* and SRD

From Table 1 it is obvious that SRD exists in *Armadillidium vulgare* (Nieuwpoort), *A. pulchellum* (Lanaken) and *Oniscus asellus* (Waasmunster), as opposed to the remainder of the populations and species included. Over the past 25 years the SRD rate in *A. vulgare* (Nieuwpoort) tended to decrease: the latest survey was even unbiased. Although the 16S rDNA of the *Oniscus asellus* F-like bacteria was not sequenced, it was considered *Wolbachia pipientis* on the grounds that the host behaved exactly like others previously disclosed to harbour *Wolbachia* (BOUCHON et al., 1998), and the bacterial cells had a typically wolbachial size and shape. SRD in *A. pulchellum* was not further studied, but it is most likely that it would be *Wolbachia*-regulated, too.

General observations as to the infection status are shown in Table 1 and Fig. 2. Note that in *A. vulgare*, as opposed to *O. asellus*, the bacteria are better traceable in the fat bodies rather than the adjacent ovaries, a conclusion consistent with that of DOBSON et al. (1999). However, especially in woodlice R gene activity can also underlie the poor, if any, conspicuousness of F within the oocytes.

Detailed external and internal morphological analyses were made of the intersexes (iF, iM and M_{og}) in *A. vulgare* and *O. asellus*. Animals of different categories were tested for cytoplasmic infection of fat bodies and reproductive organs by means of the DAPI fluorescence microscopical method. The results are schematized in Table 2. Interestingly, the fat bodies and the testes of two out of six Waasmunster *O. asellus* males harboured the feminizing endosymbiotic bacteria, albeit at visibly lower densities than females. This is concordant with the results from BOUCHON et al. (1998), where three out of ten males and six out of 13 females were positive for *Wolbachia*. It may seem quite astonishing that functional males be infected with feminizing *Wolbachia*; however, this condition was also observed in two other woodlouse species, *Chaetophiloscia elongata* and *Porcellionides pruinosus* (JUCHAULT et al., 1994). This implies a strong conflict between host and bacterial genomes to restore the male sex.

Fig. 3a shows a.o. the observed course of SRD over the past 25 years in Nieuwpoort *A. vulgare*. This population has a lot in common with the one from Niort in France (JUCHAULT et al., 1980a, 1992). Its 1977 SR of approximately 20% males reflects a sweep by F shortly beforehand. In the following years the SR increased gradually to just exceed 40% males by 1997. As was confirmed by experimental data (Table 2), F infection at that point was not at all that frequent any more. This implies that transmission suppressors (R) and/or male restorers (M) had arisen, whether or not along with the occasional transition of F to f and consequent partial loss of F.

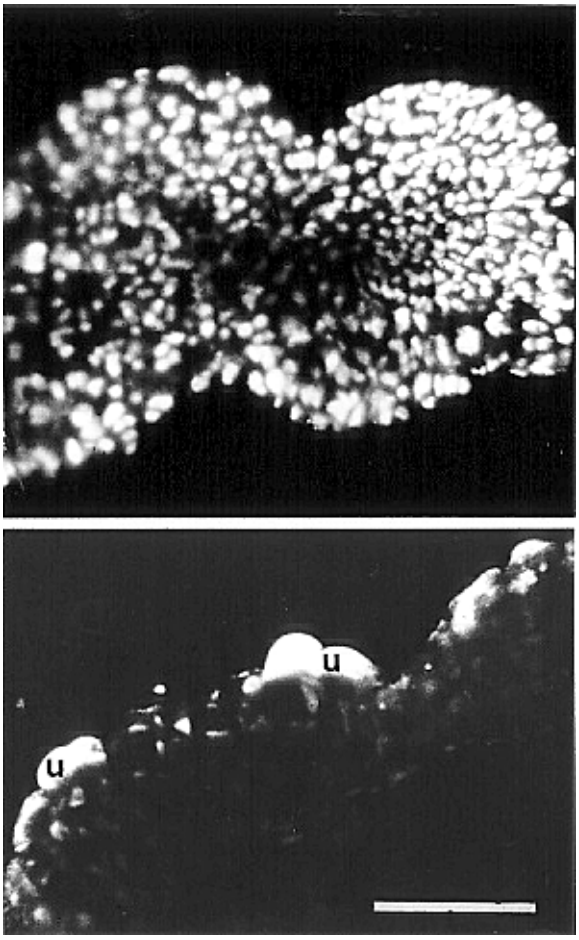

Fig. 2. – DAPI fluorescence micrographs of an uninfected (upper) and an infected (lower) *A. vulgare* fat body. u, ulcer containing numerous intracellular F bacteria appearing as intensely fluorescent clouds at low magnification. The remainder of the objects are host cell nuclei. Bar, 50 μm.

TABLE 2

(Inter)sex compositions and infection levels as determined by the DAPI assay. Fractions of examined animals that were infected by F are given between parentheses. # females excluding iF and iM; # males excluding Mog.

Species	Populations	Date	SR (%)	# females	# iF	# iM	# Mog	# males
A. vulgare	Nieuwpoort	11/1995	34.0	66 (8/18)	2 (1/2)	0	0	34 (0/9)
		11/1997	41.8	100 (3/8)	10 (1/10)	3 (1/3)	0	81 (0/3)
	Gent	11/1995	54.1	90 (0/20)	2 (0/2) †	0	0	106 (0/20)
		11/1997	48.5	67 (0/8)	2 (0/2) †	1 (0/1) †	0	66 (0/2)
O. asellus	Waasmunster	05/2000	22.9	74 (3/6)	1 (1/1)	0	0	22 (2/6)
	Gent	05/2000	43.8	9 (0/6)	0	0	0	7 (0/6)

† All F-uninfected but positive for female intersexes indicates that f is the feminizing factor in Gent; see conclusion section for further explanation on stable equilibrium SR upon recovery from infestation by F

What evidence do the 1997 and other observations provide for these conflicting factors? When carefully contemplating the crossing experiments of RIGAUD & JUCHAULT (1993), one can conclude that the intersex ratio (IR) among female phenotypes (= iF + iM / "normal" females + iF + iM) is <2% in the absence of M, whereas involvement of M in all of the crosses is associated with an IR of 26-46%. In addition, M is more frequent in F/f mixed populations (about 30%) (JUCHAULT et al., 1992) than f-only populations (<10%) (JUCHAULT & LEGRAND, 1981b) since both sexes can transmit M in the former. Extrapolating these percentages to the Nieuwpoort pill bugs in 1997, having an IR of 14% (deduced from Table 2) means that M played a role in roughly 40% of the matings. Thus, the prevalence of M can be estimated to be about 20%; in other words, the study was dealing with a mixed F/f population with preponderating f. The M_{og} phenotype as direct evidence for the M allele was not seen. M_{og} is very rare in nature indeed; even iM usually accounts for <3% of the intersex total (RIGAUD & JUCHAULT, 1993). On the other hand, R gene activity was directly evidenced by the absence of F bacteria in the gonads whereas they did populate the fat bodies.

The summary of the micro-evolutionary SRD traject of Nieuwpoort *A. vulgare* would then look like this. Beginning at a point where F fleeced the population, the R system arose. Soon after the initial transition from F to f, the origin and expansion of M was favoured in the mixed F/f population. The ensuing decrease in SRD brought about an even further reduction of F to the benefit of f, so that by 1995 the SR had gone up to 30-40% males while still growing. In 1997 the population harboured maybe more f and M than F, and the IR was typically over 10%, again in agreement with the Niort population at a corresponding stage (JUCHAULT et al., 1992).

Evolutionary ecological model

The following mathematical model intends to describe the changing SR in an *A. vulgare* population starting at a stage of infestation by F but not f. In such a population, all individuals are genetic males (ZZ) and the female sex is determined by F and f only. It was assumed that (i) neither F nor f have any fecundity effect on the isopods (demonstrated by RIGAUD et al., 1999), (ii) there is no fertility cost in phenotypic females, (iii) the woodlice reproduce once per year with discrete generations, a condition normally fulfilled in Northwestern Europe. Let F_t be the proportion of ZZ+F neo-females, and f_t the proportion of ZZ+f neo-females at time (or generation) t. Then, for the next generation, the following relationships can be expressed:

$$F_{t+1} = T_F F_t.(1 - R_t).(1 - I) \qquad (1)$$

and

$$f_{t+1} = (I.F_t + T_f f_t).(1 - M_t) \qquad (2),$$

R_t representing the instantaneous decrease in F transmission induced by the R polygenic system, I standing for the extent of integration of the wolbachial DNA segment f into the host cell nucleus before loss of the F factor, T_F and T_f being the natural transmission rates (i.e., without any counterweight on the part of the host) of F and f, respectively, and M_t the instantaneous frequency of the M allele causing a decrease in ZZ+f neo-females due to masculinization. Once R and M have originated, their frequencies can be estimated to evolve in concert with F_t and f_t, respectively:

$$R_t \cong I_F^t .(F_0 - F_t) \qquad (3)$$

and

$$M_t \cong T_f^t .(f_t - f_0) \qquad (4).$$

However, substituting (3) and (4) into (1) and (2) yields an unrealistic model in which all situations lead to a heavily male-biased outcome within few generations. Likewise, neo-females are quickly outnumbered if T_F or T_f are not equal to 100%, despite previous estimates for both not exceeding 85% (RIGAUD et al., 1992; but see remarks below). Partial replenishment of the female sex could be accounted for by introgression of (neo-)females from neighbouring populations, but in the case of the fairly isolated Nieuwpoort population this can hardly suffice to warrant persistence of neo-females at the levels observed. Apparently, other conditions are needed to explain the continuity of F/f in Nieuwpoort *A. vulgare*. It is not exceptional to find transovarial transmission rates of 100% among F-like bacteria, e.g. in parasitoid wasps (HUIGENS et al., 2000) and dagger nematodes (COOMANS et al., 2000; VANDEKERCKHOVE et al., 2002). There is reason to believe

that the above T_F of 0.85 is not the natural F transmission rate: on the one hand because it was derived from artificial – not wild – populations stemming from a selection of ten year inbred ZZ+F lines (these were known to lack M, though more importantly, absence of R was not guaranteed), and on the other hand because it is actually termed "primary transmission rate" and represents the aggregate of most mothers transmitting at virtually 100% and a few with very weak transmission (RIGAUD et al., 1992). The latter could be elicited by switching on R expression, and then 0.85 must not be considered the natural F transmission rate indeed. Likewise, the primary T_f would vary a great deal between 0.53 and 0.80 in an artificial ZZ+f population, but a natural T_f close to 1.00 is expected if the transposable element f copies itself without hindrance to several other loci in the host genome. Therefore, we do let T_F and T_f approximate 1.00 in (3) and (4). Moreover, we let R_t and M_t depend differently upon F_t and f_t to obtain the new relationships:

$$R_t \cong F_t - F_{t+1} \qquad (5)$$

and

$$M_t \cong f_{t+1} - f_t \qquad (6).$$

These assumptions can be made on the grounds that R and M are the principal factors responsible for the sink in ZZ+F and ZZ+f neo-females, respectively. The spread of R lags behind as compared to the behaviour of F, whereas M evolves ahead of *f*, thereby determining much of the ZZ+f increase to the detriment of F. Substitution of equation (5) into (1), and (6) into (2) yields, upon rearrangement:

$$F_{t+1} = \frac{1 + (I-1).F_t^2}{1 + (I-1).F_t} - 1 \qquad (7)$$

and

$$f_{t+1} = \frac{T_f.f_t^2 + (T_f + I.F_t).f_t + I.F_t}{1 + I.F_t + T_f.f_t} \qquad (8),$$

so that finally, the SR in generation t+1 can be expressed as the fraction of males by subtracting the fractions of both classes of neo-females from 1:

$$SR_{t+1} = 1 - (F_{t+1} + f_{t+1}) \qquad (9).$$

In 1977 (t = 1) the Nieuwpoort *A. vulgare* SR was calculated to be 22.0% but in reality it was probably lower since two years later it was as low as 16.9%. Therefore, we let e.g. SR_0 = 10%; this means $F_0 + f_0 = 0.90$. In view of the very low SR, most of the females at that time must have been ZZ+F with only a minor fraction being ZZ+f. As such, $F_0 = 0.82$ and $f_0 = 0.08$ would be good starting values to enter, but even if we let F_0 vary between 0.60 and 0.90 (f_0 between 0.00 and 0.30) this has only a minor influence on the issue (Fig. 3a). The same is true for SR_0 (Fig. 3a). Thus, the parameters of importance are *I* and T_f.

The predictions from the model with a range of values for *I* and T_f are summarized in Figs 3b and 3c. *I* is expected to be very low: when the wolbachial DNA segment f is incorporated into the host nucleus, the bacterium itself usually remains and overrules f as long as R is not involved to reduce the vertical transmission of F. The higher *I*, the sooner the equilibrium SR is attained (Fig. 3b). Further-

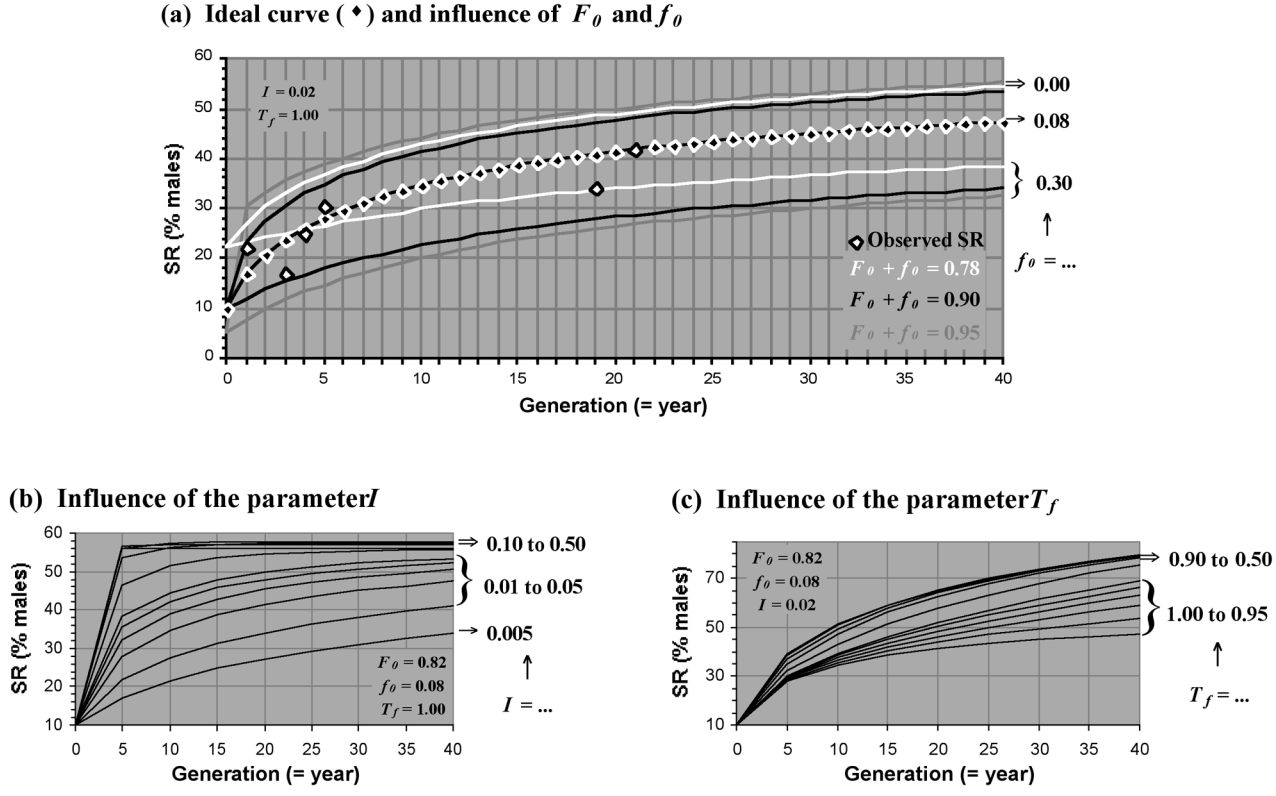

Fig. 3. – Behaviour and predictive power of the deterministic model (described by equations 7-9 in this report) under a range of realistic circumstances.

more, $T_f = 1.00$ yields results that harmonize with the reality of the Nieuwpoort pill bugs. $T_f < 1.00$ (e.g., if f remains a low-copy transposable element) causes an enormous acceleration of the SR evolution with extensive masculinization (Fig. 3c), consistent with the experiments of RIGAUD et al. (1992). It should be pointed out, though, that the present model loses in accuracy under accelerated circumstances. On the whole, $T_f < 0.85$ is attended with relative persistence of ZZ+F instead of ZZ+f neo-females regardless of the presence of R. In the special situation where $0.85 < T_f < 1.00$, ZZ+f neo-females gain in numbers for up to several tens of generations and seem to hold quite comfortably, but this is a pseudo-equilibrium: they fade away eventually after a few hundreds of generations. By that time the whole population would have become male, and have disintegrated consequently.

Altogether, there are several rather well-fitting parameter combinations, the best values being also realistic ones, including the natural transmission rates of F and f that may indeed equal 1.00. This proves the applicability of the deterministic model under the given circumstances in the field. Nevertheless, an inconsistency can be found in that the theoretical proportion of ZZ+F neo-females after 21 generations (using the parameter values from the "ideal curve" in Fig. 3a) equals 33.3%, implying that *Wolbachia* should have been discovered in about 50% of the phenotypic females, whereas the DAPI assay diagnosed only five positive cases out of 21 (Table 2). This is thought to result from overlooking poor infection levels. By contrast, half of the Waasmunster *O. asellus* females were infested, indeed (Table 2).

Outcome of the model – conclusions

What does the model predict for the further evolution of the SR in Nieuwpoort *A. vulgare*? Let us take a look at the following data, computed upon incorporation of the "ideal curve" parameters from Fig. 3a: $SR_{100} = 52.6\%$, $F_{100} = 5.0\%$, $f_{100} = 42.4\%$; and still further: $SR_{200} = 54.0\%$, $F_{200} = 0.6\%$, $f_{200} = 45.4\%$. Clearly, there is a tendency for f_t to increase along with SR_t while F_t decreases continually. It can be proved that the limits for SR_t, F_t and f_t are reached after 326 generations and equal 56.0%, 0.0% and 44.0%, respectively. In such a population, the numbers of both sexes are approximately alike but all females are genetic males carrying the f segment and not M. Most males also carry f, but it is overridden by M which nonetheless enables its transmission to offspring. An *A. vulgare* population where all individuals are genetic males with M and f as principal sex determinants can most probably be found in Ghent: here, the pill bugs still show vestiges of intersexes but not a single specimen was infected by F (Table 2), a status reconcilable only with f's prevalence at a stable equilibrium SR of theoretically 56% (already reached in 1995 – see Table 1). In other words, the chromosomal sex determination system has switched from W/Z to f/M. CAUBET et al. (2000) illustrated nicely how such switches can occur consecutively under external forces by which the sex of organisms is manipulated uninterruptedly. The other F-negative entries in Table 1 were not further investigated, but it is not impossible that some of them share this property with *A. vulgare* from Gent.

The power of this model lies in its simple truth to nature and its flexibility. It describes how *Wolbachia* can either persist for a prolonged period or in many instances be driven out systematically in the long term, often but not always taken over by the wolbachial segment f, the latter being less strongly feminizing in that it is overruled by the masculinizing factor M though taking advantage of the latter's favourable company through the hitchhiking effect (MAYNARD-SMITH & HAIGH, 1974). Hence, it appears that fixation of f is easier than that of F. Similar conclusions were drawn by JUCHAULT et al. (1992) and RIGAUD et al. (1992).

More concretely, this model emphasizes that: (i) SRD evolution is at least in *A. vulgare* reduced to a subtle game between transition (= *I* throughout this report) of *Wolbachia* to a transposable element and the transmissible copy number (= transmission potential, or T_f) of the latter in the host genome; (ii) this f transmission rate is decisive as to whether ZZ+f females will thrive and attain an (either stable or pseudo-) equilibrium, or go extinct quicker than F, with a critical T_f for pseudo-maintenance around 0.85; (iii) the F-to-f transition rate matters mainly to the speed with which the end condition is reached, not to this condition itself; (iv) the initial abundances of both *Wolbachia* (= F_0) and the wolbachial transposon (= f_0) have only a slight influence on the outcome of SR evolution.

The weakness of the model is its inaccurate representativeness in situations where the transmission potential of the wolbachial transposon deviates significantly from 100%, or more generally, under accelerated evolutionary circumstances: the terror of many mathematical models. Besides, the course of an SR curve – however determinative or predictive it may seem – can be disrupted at any point in time by a new invasion, e.g. by a *Wolbachia* strain resistant to the R genes. The ensuing arms race would then comply with entirely different mathematics until the sweep is near completion. By no means do the formulae introduced here advocate a neatly ordered series of events in infected isopods, yet it would be rewarding to see if they can be successfully applied to other populations or further refined from studies of other species (see Table 1 for some good candidates).

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APPENDIX

The Turbo Pascal program implementing the mathematical model in this study. Note that syntax rules do not allow every variable and parameter denomination to be the same as in the discussion text.

```
program WOODLICE (input, output);
uses crt;
var
  Bo, Bt, fo, ft, I, Tf, SEXRAT_M : real;
  Z, ITERATIO : integer;
begin
  clrscr;
  writeln ('  Deterministic model describing sex ratio');
  writeln ('   changes in Wolbachia-infested woodlice');
  writeln;
  writeln ('-----------------------------------------------');
  writeln ('Ft = Fo.(1 - Rt).(1 - I)      [Rt = Fo – Ft]');
  writeln;
  writeln ('ft = (I.Fo + Tf.fo).(1 - Mt)  [Mt = ft – fo]');
  writeln;
  writeln ('SRt = 1 – (Ft + ft)');
  writeln ('-----------------------------------------------');
  writeln;
  writeln ('Enter the values for parameters I and Tf successively: ');
  read (I, Tf);
  writeln ('How many iterations? : ');
  read (ITERATIO);
  writeln ('Start values for Fo and fo? : ');
  read (Bo, fo);
  writeln;
  writeln ('Sex ratio / Ft / ft after iteration');
  for Z := 1 to ITERATIO do
    begin
      ft := (Tf*sqr(fo)+(Tf+Bo*I)*fo+Bo*I)/(1+Bo*I+Tf*fo);
      Bt := ((1+(I-1)*sqr(Bo)/(1+(I-1)*Bo))-1;
      SEXRAT_M := 1-Bt-ft;
      write (Z);
      write (' : ');
      writeln (SEXRAT_M:3:3, '   ', Bt:3:3, '   ', ft:3:3);
      fo := ft;
      Bo := Bt
    end;
  repeat until keypressed
end.
```

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