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CHAPTER **11**

Onchocerca–Simulium Interactions and the Population and Evolutionary Biology of *Onchocerca volvulus*

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Abstract

Parasite–vector interactions shape the population dynamics of vector-borne infections and contribute to observed epidemiological patterns. Also, parasites and their vectors may co-evolve, giving rise to locally adapted combinations or complexes with the potential to stabilise the infection. Here, we focus on *Onchocerca–Simulium* interactions with particular reference to the transmission dynamics of human onchocerciasis. A wide range of simuliid species may act as vectors of *Onchocerca volvulus*, each exerting their own influence over the local epidemiology and the feasibility of controlling/eliminating the infection. Firstly, current understanding of the processes involved in parasite acquisition by, and development within, different *Simulium* species in West Africa and Latin America will be reviewed. A description of how *Onchocerca* and *Simulium* exert reciprocal effects on each other's survival at various stages of the parasite's life cycle within the blackfly, and may have adapted to minimise deleterious effects on fitness and maximise transmission will be given. Second, we describe the interactions in terms of resultant (positive and negative) density-dependent processes that regulate parasite abundance, and discuss their incorporation into mathematical models that provide useful qualitative insight regarding transmission breakpoints. Finally, we examine the interactions' influence upon the evolution of anthelmintic resistance, and conclude that local adaptation of *Onchocerca–Simulium* complexes will influence the feasibility of eliminating the parasite reservoir in different foci.

11.1. INTRODUCTION

Understanding the interface between parasites and their hosts provides insight into the biology of the infection process and how this process contributes to shape interactions at the population level. Also, this interface provides the backdrop for selection as at various stages in the interaction there will be pressures on parasites to enhance their transmissibility, which may result in them manipulating or exploiting the interaction and in hosts responding in various ways that help fend-off infection, and/or minimise damage or deleterious effects on fitness. Host–parasite interactions, therefore, have the potential to contribute to the shaping of short- and long-term epidemiological patterns, parasite–host co-evolution, and evolutionary change under selection pressures exerted by control interventions. These interactions will take place at various scales, from the molecular and cellular levels to the organismal and population levels. The detection of organismal- and population-level interactions (as emergent properties) can inform research into the molecular and cellular underlying mechanisms.

In this review we focus on the manifestations of *Onchocerca–Simulium* interactions as density-dependent effects, acting upon the parasite infrapopulation within the flies and upon the flies themselves. We examine evidence to suggest that *Onchocerca* may exploit such interactions to enhance its transmission and discuss the consequences on onchocerciasis transmission of local adaptation in *Onchocerca–Simulium* complexes. Finally, we examine the consequences of these processes for the population biology, transmission dynamics and control of *Onchocerca volvulus*.

11.1.1. *Onchocerca–Simulium* complexes

The interactions between the spirurian nematode parasites of the genus *Onchocerca* (Filarioidea: Onchocercidae) and their haematophagous intermediate hosts have been most extensively investigated for *Onchocerca volvulus* Leuckart in its *Simulium* Latreille (Diptera: Simuliidae) vectors. It is in the context of human onchocerciasis that the term *Onchocerca–Simulium* complexes was first used by Duke *et al.* (1966) to refer to well-adapted parasite–vector combinations that result in the development and transmission of the local *O. volvulus* population (e.g., the savannah form of the parasite developing successfully within savannah species of *S. damnosum* Theobald *sensu lato* (s.l.) but less so within forest species, and vice versa). Through a series of cross-experimental infection studies, in which flies were fed on microfilarial carriers of the same (sympatric) and distant (allopatric) localities, investigation of such complexes encompassed *O. volvulus–Simulium* comparisons not only within West Africa

but also between West Africa and Guatemala (De Leon and Duke, 1966; Duke *et al.*, 1967), West Africa and northern Venezuela (Duke, 1970), Guatemala and northern Venezuela (Takaoka *et al.*, 1986a,b), and more recently between the northern and Amazonian foci within Venezuela (Basáñez *et al.*, 2000). The results suggest the operation of strong local adaptation between the parasite and its vectors within well-established endemic areas, as opposed to greater incompatibility in heterologous combinations. The relevance of these findings is two-fold; first, because they have important implications for our understanding of the potential for onchocerciasis to spread outside its current endemic areas (Basáñez *et al.*, 2000; Maia-Herzog *et al.*, 1999; Schiller *et al.*, 1984), and second because they raise the question as to what would have been the potential for invasibility of New World areas, with local anthropophagic simuliid fauna and unexposed human populations, by *O. volvulus* when first brought to the Americas from Africa during the slave trade. (For the concepts of species invasiveness and community invasibility we follow Richardson and Pyek, 2006.)

11.1.1.1. Vector complexes

The notion of *Onchocerca–Simulium* complexes shall not be confused with the concept of vector species complexes, of which *S. damnosum* s.l. is an example (Vajime and Dunbar, 1975). A species complex (or a complex of 'sibling' or 'cryptic' species) is a group of closely related species, which, although may satisfy the criterion of being reproductively isolated from each other, are not readily or reliably distinguishable on a morphological basis, necessitating the use of cytological, genetic and/or ecological attributes to distinguish between them (White, 1978). Chromosomal speciation is widespread in Simuliidae (Rothfels, 1989). There are 55 valid and distinct cytoforms known from the *S. damnosum* complex, making it the largest sibling species complex (Post *et al.*, 2007). Nine sibling species serve as vectors for *O. volvulus* in West Africa, albeit with different capacities (Boakye, 1993). These include a savannah-dwelling group (*S. damnosum* sensu stricto and *S. sirbanum*; Vajime & Dunbar), a forest-dwelling group (*S. squamosum* Enderlein and *S. yahense* Vajime & Dunbar) and a transition-zone (forest-savannah mosaic)-dwelling group (*S. sancti-pauli* Vajime & Dunbar, *S. leonense* Boakye, Post & Mosha, and *S. soubrense* Vajime & Dunbar) (Boakye *et al.*, 1998). Cytogenetic (Boakye, 1993) and DNA sequence analyses (Tang *et al.*, 1995) have supported the division of the sibling species into these three groups, and the former has indicated that hybridisation between the siblings and introgression occur (Boakye and Meredith, 1993; Boakye and Mosha, 1988; Boakye *et al.*, 2000). For a discussion of vector complexes in Latin America, see Shelley (1991). Fig. 11.1 presents a map of Africa (with Latin America inset) showing
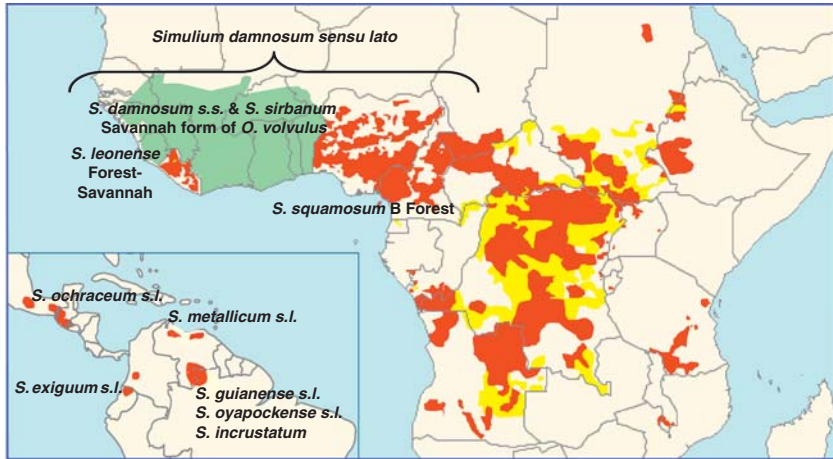
FIGURE 11.1   Distribution of human onchocerciasis endemic areas and vector species discussed in the text. The medium grey-shaded area represents the Onchocerciasis Control Programme in West Africa (OCP, 1975–2002); the dark grey areas depict regions presently undergoing mass ivermectin administration (annually in Africa, African Programme for Onchocerciasis Control (APOC, 1995–on-going) and biannually in Latin America, Onchocerciasis Elimination Program for the Americas (OEPA, 1993–on-going)); light grey areas require further rapid epidemiological mapping for onchocerciasis (REMO) surveys. Adapted from Basáñez *et al.* (2006).

the distribution of onchocerciasis, its control programmes and indicating the main vector species that will be discussed in this review.

11.1.2. *Onchocerca* in the Simuliid host

There are many opportunities for interaction between *Onchocerca* and its *Simulium* vector as the parasite undergoes considerable growth and development within the fly's thoracic muscles. Microfilariae must first be ingested with the blood meal, and a substantial portion of the blackfly's mouthparts is introduced into the skin of the definitive host by the cutting and piercing action of the mandibles and maxillae. Fig. 11.2 summarises the main stages in the process of infection at which the parasite encounters an interface with the vector which presents a challenge and an opportunity for interaction.

11.1.2.1. Ingestion of microfilariae

Contact between *Simulium* and *O. volvulus* begins with the ingestion of a blood meal containing microfilariae (the stage infective to the vector). When feeding, female simuliids locate the blood by cutting the skin of
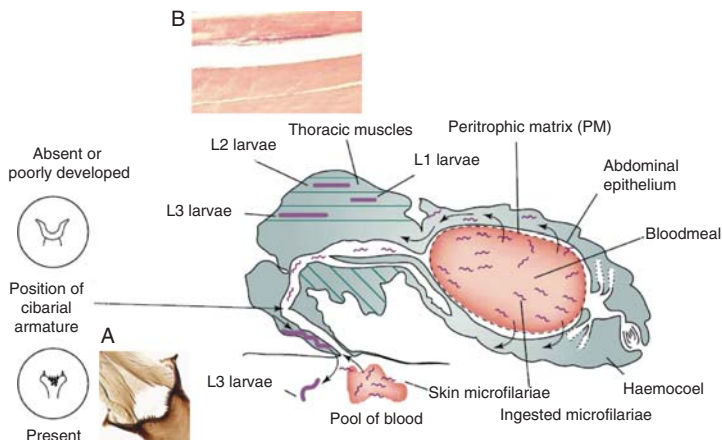
FIGURE 11.2   *Onchocerca* in the *Simulium* host. Microfilariae are recruited to the site of the wound caused by the piercing and cutting blackfly's mouthparts, and ingested with the blood meal (salivary secretions by the fly act on the vertebrate's haemostatic system and may attract microfilariae). Well-developed cibarial armatures (A) may cause lacerations to microfilariae, which otherwise must reach the haemocoel escaping imprisonment by the peritrophic matrix (PM). In the thoracic muscles (B), development of established microfilariae takes place to the infective stage in competent vectors. (A) Cibarium of *S. incrustatum* from the Brazilian part of the Amazonian focus; photograph taken by Luis Hernández, Natural History Museum, London. (B) Microfilariae of *O. volvulus* in the thorax of *S. metallicum* s.l. from north Venezuela; photograph taken from the archive of Jaime Ramírez-Pérez by Edmundo Guerrero, Tropical Zoology Institute, Universidad Central de Venezuela. Adapted from Basáñez and Ricárdez-Esquinca (2001).

the vertebrate host with rapid scissor-like movements of their mandibles, their maxillary laciniae penetrating downwards and creating a haemorrhagic pool upon which they feed (Ayesta *et al.*, 1985; Sutcliffe and McIver, 1984). While the hard mouthparts lacerate the skin, the membranous cuticles prevent, mechanically, blood loss from the wound and air entry into the food canal during feeding. The labral flaps within the food canal act as one-way valves, keeping blood from leaking out during the pumping down-stroke but allowing pooled blood to enter during the upstroke (Sutcliffe, 1985).

Simultaneously during this process, blackfly's saliva containing anti-haemostatic, pro-inflammatory, erythema-inducing, and immunomodulatory molecules (such as apyrase, anti-coagulants and anti-thrombin salivary protein) is injected into the host's skin at the site of the bite. These substances not only inhibit platelet aggregation, reduce coagulation

and induce vasodilation increasing blood supply to the feeding wound (facilitating easier and longer feeding), but the saliva's ability to modulate components of the host's immune system also provides an opportunity for enhanced parasite transmission during blood feeding (Andrade *et al.*, 2005; Cupp and Cupp, 1997; Hurd, 2003). Skin-dwelling *O. volvulus* microfilariae migrate towards the small pool of blood before being ingested by the blackfly and experimental evidence suggests that microfilarial orientation, movement and concentration into the feeding site may be mediated by a protein in the insect's saliva different from the erythema-inducing protein described earlier for New World simuliids (Stallings *et al.*, 2002). Although the hypothesis of whether *O. volvulus* could exploit the nature and activity of blackfly's salivary secretions to enhance its transmission must still be rigorously tested, in the following section the feeding experiment results that suggest this indeed might be an adaptive strategy in the *Onchocerca–Simulium* interaction will be summarised.

Cupp *et al.* (1995) reported that although apyrase activity inhibits platelet aggregation and is ubiquitous in blackfly's saliva, activity per gland varied according to species and had a positive association with degree of anthropophagy and *O. volvulus* vector status, being higher in *S. ochraceum* Walker than in *S. metallicum* Bellardi and *S. exiguum* Roubaud, with the lowest levels exhibited by the non-vectors *S. bivittatum* Malloch and *S. gonzalezi* Vargas & Díaz-Nájera. By contrast, anti-thrombin presence and activity also varied among blackfly species but exhibited a positive correlation with zoophagy (Cupp and Cupp, 1997). These authors also demonstrated that various simuliid species in the American continent differ in the vasodilator activity of their salivary secretions, with *S. ochraceum* s.l. exhibiting higher levels of such activity than *S. metallicum* s.l. Prior to this, working in Guatemala, Omar and Garms (1975) had recorded higher microfilarial intakes by *S. ochraceum* s.l. than by *S. metallicum* s.l., and De Leon and Duke (1966) had shown that microfilarial intake by *S. ochraceum* s.l. was also higher than that by *S. callidum* Dyar & Shannon. Shelley (1988), working in Ecuador, suggested that *S. quadrivittatum* Loew ingested more microfilariae than *S. exiguum* s.l., and Grillet *et al.* (2008) found, in the Amazonian focus of Venezuela, that *S. oyapockense* Floch & Abonnenc s.l. was able to ingest more microfilariae than *S. incrustatum* Lutz given the same microfilaridermia. These results suggest an inherent difference in the ability of various *Simulium* species to acquire microfilariae.

11.1.2.2. Survival of the microfilariae

The *O. volvulus–Simulium* interaction continues as blood is pumped into the stomach. In those species with a well-developed cibarial (sometimes referred as buccopharyngeal) armature, microfilariae may encounter rows of chitinous projections that protrude into the lumen of the foregut

as they are ingested together with the blood meal through the action of the cibarial and pharyngeal pumps located in the blackfly's head (Sutcliffe and McIver, 1984). Depending on the morphology of the armature, the projections may be more or less numerous, arranged in single or multiple rows, and blunter (papillae, rods) or sharper (cones, spines) in shape— there possibly is intra- as well as inter-specific variation in the armature's shape and size. In the context of onchocerciasis transmission in Africa and Latin America, two distinct groups of species are recognised according to the presence or absence of a well-developed armature (Basáñez and Ricárdez-Esquinca, 2001; Reid, 1978, 1994; Shelley, 1988). The former comprises species with cibarial 'teeth', including *S. ochraceum* s.l. and *S. haematopotum* Malloch in Meso American foci; *S. oyapockense* s.l., *S. incrustatum*, and *S. limbatum* Knab in the Amazonian focus, and *S. quadrivittatum* in Ecuador. The latter comprises species with poorly developed armatures, including the *S. damnosum* complex and the *S. neavei* Roubaud group in Africa, and *S. metallicum* s.l., *S. exiguum* s.l. and *S. guianense* Wise s.l. in Latin America.

Since cibarial armatures are more developed in the haematophagous females than in the non-blood-sucking males of certain families of biting flies, including Simuliidae, it has been proposed that the armature has mainly evolved in response to the blood-feeding habit, partly acting to prevent blood back-flow, and partly involved in the breaking up of erythrocytes to release the haemoglobin and other proteins prior to digestion of the blood in the abdominal mid-gut (Reid, 1994). However, secondarily and in terms of resistance to infection and simuliid survival, the cibarial armature serves as a first line of defence against ingested microfilariae (and parasite-induced fly mortality) by inflicting lacerations on the invading microfilarial stage.

Omar and Garms (1975) were the first to describe the consequences of the cibarial armature (of *S. ochraceum* s.l. in Guatemala) upon microfilariae of *O. volvulus* in contrast to the effect of an unarmed cibarium (of *S. metallicum* s.l.) and concluded that the armature damaged a substantial proportion of microfilariae en route to the stomach, where they disintegrated. Only 3% of the microfilariae migrated towards the thorax in *S. ochraceum* s.l. in comparison with 75% in *S. metallicum* s.l. (despite the former having ingested a significantly higher number of microfilariae than the latter). Omar and Garms' findings thus explained earlier observations by Bain *et al.* (1974) on the existence of 'two populations of microfilariae', one with the ability to migrate out of the stomach while the other is rapidly destroyed in an inverse proportion to the number of microfilariae ingested. Basáñez *et al.* (1995) suggested that Bain's observations could be explained by arguing that the fraction of microfilariae damaged by the cibarial armature could be density dependent, with a higher probability that ingested parasites are lacerated by the cibarial

teeth at lower intakes, and a smaller probability of damage at higher intakes (when some microfilariae may be protected by those that become entangled in the cuticular projections). Recent data from other 'armed' species (e.g., *S. oyapockense* s.l. and *S. incrustatum* in the Amazonian onchocerciasis focus) lend support to this hypothesis and indicate that the average damage caused is species specific (Grillet *et al.*, 2008). Interspecific variations in the shape of the cibarial armature and how these differences may be reflected in the degree of damage produced upon ingested filarial parasites have been observed in mosquitoes (McGreevy *et al.*, 1978). Table 11.1 presents a functional classification of simuliid vectors according to the presence/absence of a well-developed cibarial armature. All those (natural) armed vectors of human onchocerciasis are only in the Americas.

The fact that the cibarial armature, when well developed, can substantially reduce the number of microfilariae that are available for further migration to the thoracic muscles of the vector raises the question as to whether the parasite can overcome this constraint by exploiting the vector–parasite interaction to enhance other aspects of transmission. In the previous section we described the anti-haemostatic, anti-coagulant and vasoactive properties of blackfly's saliva on the host's blood (Cupp and Cupp, 1997). It is also possible that the parasite may utilise these properties of the vector–parasite interface to increase microfilarial intake by those simuliid species that destroy ingested parasites with their cibarial armatures. This could partly explain the results of the pair-wise comparisons listed above, in which the first species of the pair has been reported to exhibit higher microfilarial intakes than the second, namely, *S. ochraceum* s.l. (armed) versus *S. metallicum* s.l. (unarmed) (Omar and Garms, 1977); *S. quadrivittatum* (armed) versus *S. exiguum* s.l. (unarmed) (Shelley, 1988); *S. oyapockense* s.l. (more damaging armature) versus *S. incrustatum* (less damaging) (Grillet *et al.*, 2008).

11.1.2.3. Passage of microfilariae out of the blood meal

Lewis (1953) clearly described that the microfilariae of *O. volvulus* do not bypass an abdominal phase by reaching the thoracic muscles through direct migration from the thoracic mid-gut en route to the stomach. Rather, once in the abdominal mid-gut, microfilariae must leave the blood meal, reach the ecto-peritrophic space, traverse the abdominal epithelium, reach the haemocoel and migrate to the thorax, where they penetrate the muscle fibres and eventually grow and moult twice to become infective, L3 larvae. This abdominal phase therefore represents the next opportunity for *Onchocerca–Simulium* interaction. In response to the blood being ingested, a thick extracellular matrix (peritrophic matrix (PM), first described by Lewis (1950) for *S. damnosum*) is secreted (delaminated) by the abdominal mid-gut epithelium, completely surrounding

TABLE 11.1 Simuliid hosts of *Onchocerca* classified according to the presence or absence of a well-developed cibarial armature, their vector status and parasite density dependence

Locality	Type of cibarial armature[a] Well developed	Poorly or not developed	Vector status[c]	Relationship between input microfilariae and output larvae[d]	References
West African savannah		*Simulium damnosum* s.s./ *S. sirbanum*	Natural	Limitation (negative density dependence)	Basáñez *et al.*, 1995; Philippon and Bain, 1972
West African forest (incl. forest-savannah mosaic)		*S. leonense*	Natural	Proportionality (density independence)	Soumbey-Alley *et al.*, 2004
		S. squamosum B[b]	Natural	Proportionality	Demanou *et al.*, 2003
East Africa		*S. neavei*	Natural	N/A	
Mexico and Guatemala		*S. callidum*	Natural	N/A	Dalmat, 1955
	S. haematopotum		Experimental	N/A	Takaoka *et al.*, 1986a
		S. metallicum A–K, X[b]	Natural	N/A	Shelley, 1991
	S. ochraceum A–C[b]		Natural	Initial facilitation (positive density dependence)	Basáñez *et al.*, 1995; Collins *et al.*, 1977
	S. veracruzanum		Experimental	N/A	Shelley, 1991
Colombia		*S. exiguum* s.l.	Natural	N/A	Tidwell *et al.*, 1980; Wetten *et al.*, 2007

Ecuador		==*S. exiguum* Cayapa[b]==	Natural	Limitation	Collins *et al.*, 1995; Wetten *et al.*, 2007
		S. exiguum Aguarico[b] Bucay[b] Quevedo[b]	Experimental	N/A	Shelley *et al.*, 1989, 1990; Wetten *et al.*, 2007
	S. quadrivittatum		Natural	N/A	Vieira *et al.*, 2005
Venezuela (North)		*S. metallicum* E[b]	Natural	Limitation	Basáñez *et al.*, 2000; Grillet *et al.*, 1994
Venezuela and Brazil		==*S. guianense* s.l.==	Natural	Limitation	Basáñez *et al.*, 1995
(Amazonian focus)	*S. incrustatum* / *S. limbatum*		Natural	Initial facilitation	Grillet *et al.*, 2008
	S. oyapockense / *S. roraimense*		Natural	Initial facilitation	Grillet *et al.*, 2008
Britain	*S. ornatum* s.l.		Natural	N/A	Reid, 1994
		S. lineatum	Experimental	N/A	Reid, 1994

Notes: [a] According to Reid (1994);
[b] letters/names following species are cytoforms;
[c] species of the *S. damnosum* complex are also natural hosts of *O. ochengi*; *S. ornatum* s.l. is a natural vector of *O. lienalis*;
[d] input microfilariae can be microfilariae/mg of skin or microfilariae ingested/fly; output larvae can be exo-peritrophic and thoracic microfilariae or infective, L3 larvae.

the blood meal (Ramos *et al.*, 1994) and constituting a type-1 PM (Lehane, 1997). The PM is composed of proteins, glycoproteins and chitin in a proteoglycan matrix, and among its main functional roles are: 1) partition of the mid-gut lumen into physiologically meaningful compartments, such as separation of the ingested blood from the mid-gut epithelium, with the PM delimiting the gut lumen into a wholly enclosed endo-peritrophic space and an outer ecto-peritrophic space; 2) regulation of digestion and of the passage of molecules between different mid-gut compartments; 3) protection of the mid-gut cells from mechanical abrasion by blood components (e.g., sharp-edged haemoglobin-like crystals) and 4) defence against infection by pathogens and parasites (Lehane, 1997; Ramos *et al.*, 1993, 1994; Shao *et al.*, 2001).

In the context of *Onchocerca–Simulium* interactions, the several molecular, biochemical and physical properties of the PM, determining its rate of synthesis, composition, structure, thickness and degradation time, can be related to the ability of microfilariae to survive and migrate successfully towards the thoracic muscles. In blackflies, the PM starts forming within minutes of blood ingestion, with an initially rapid secretion of PM material from the mid-gut epithelium. This secretory phase is followed by a period of organisation, maximum thickness being achieved between 6 and 12 h post-engorgement (PE) depending on species. The rate of secretion, the level of organisation into distinct (or not) laminae, the resulting morphological appearance, and the final thickness of the PM appear to be species specific (Reid and Lehane, 1984). It has been suggested that only those microfilariae that penetrate the PM during the initial secretion period, before it has condensed and hardened into a distinct structure, or that find themselves adjacent to very thin areas within the matrix, may reach successfully the haemocoel of the blackfly en route to the thorax (Ramos *et al.*, 1994; Reid and Lehane, 1984). Those that do not traverse the abdominal epithelium in time, share the fate of the blood meal and are eventually digested, not contributing to transmission (Lewis, 1953).

11.1.2.4. Vector survival

Lewis (1953) and Omar and Garms (1977) described, respectively, for *S. damnosum* and *S. metallicum*, that in those flies that ingested large numbers of microfilariae, the excessive number of parasites interrupted the formation of the PM and disrupted the normal process of blood digestion, rapidly reaching many organs other than the stomach thus invading and injuring, among others, the foregut, the hindgut, the Malpighian tubules and the haemocoel. This generalised infection led to the death of the insect within a few hours PE. Given the importance that vector survival, until completion of the extrinsic incubation period of the parasite and beyond, has in the transmission of vector-borne infections

(Macdonald, 1957), Basáñez *et al.* (1996) compared mortality rates and expectation of infective life among blackfly species with (*S. ochraceum* s.l.) and without (*S. damnosum* s.l., *S. guianense* s.l.,) well-developed cibarial armatures. In all three species there was evidence of senescence (mortality rates increased with time PE, a proxy for fly's age), and of vector survival being adversely affected by increasing microfilarial load. However, the proportion of flies surviving beyond the maturation period of *O. volvulus* within the fly was higher in armed than in unarmed flies for a given microfilarial intake, suggesting that the cibarial armature affords a certain degree of protection against the damage that high numbers of ingested parasites may cause to the simuliids.

11.1.3. Aims and objectives of this review

Having summarised the various stages in the *Onchocerca–Simulium* interaction, we now proceed to review the methods that have been used and the results that have been obtained when the population consequences of such interaction have been investigated. As population regulation requires the operation of density-dependent processes, we focus on the identification and quantification of these, and their incorporation into the life cycle of *O. volvulus* so that their epidemiological end evolutionary implications can be discussed. We examine available evidence of local adaptation in *Onchocerca–Simulium* complexes and of the parasite responding to selective pressures by exploiting the interaction to maximise its transmission. Finally, we examine the consequences of these processes for the control of human onchocerciasis.

11.2. METHODS

11.2.1. A statistical description of *Onchocerca–Simulium* interactions

The quantitative relationships between consecutive *Onchocerca* stages within *Simulium* have been investigated by fitting appropriate statistical models to parasite density data obtained from series of fly-feeding experiments. The results suggest that many processes involved in the parasite–vector interaction are density dependent, requiring the use of non-linear regression methods or appropriate transformation of variables for linear analysis. In this section we describe briefly the statistical methods used, referring the reader to appropriate literature for detailed explanations that are beyond the scope of this paper.
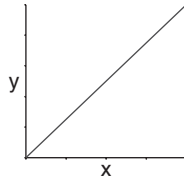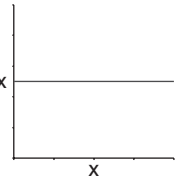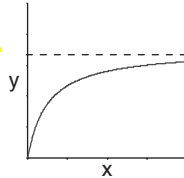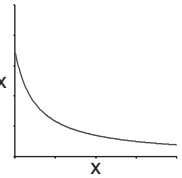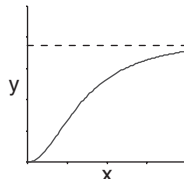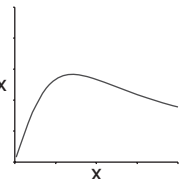
11.2.1.1. Density dependence

In the context of *Onchocerca–Simulium* interactions a biological process is density dependent (non-linear) when the per capita rate at which such process occurs (e.g., microfilarial intake for a given microfilaridermia; fraction of microfilariae damaged by the cibarial armature for a given microfilarial intake; development of microfilariae to the infective stage for a given microfilarial load, etc.) decreases or increases with parasite density. If the rate remains constant it is said to be density independent. In the literature of filariasis–mosquito interactions, the concepts of *limitation*, *facilitation* and *proportionality* have been introduced to describe, respectively, the operation of negative density dependence, positive density dependence and density independence (Pichon, 1974). The per capita rate of successful passage or development to the L3 stage has been referred to as *parasite yield* (Pichon *et al.*, 1974). Table 11.2 (adapted from Sinden *et al.*, 2007) illustrates the possible functional relationships between two consecutive parasite stages within the vector and their corresponding per capita rates or probabilities of success.

11.2.1.2. Parasite distributions and statistical consequences

The distribution of microfilariae in the human host's skin, of ingested microfilariae per fly, and of thoracic and infective larvae among vectors are all highly over-dispersed (i.e., parasites are not randomly distributed, with most hosts or vectors harbouring low parasite loads and few hosts or flies being heavily infected). The statistical study of the relationships between *Onchocerca* stages necessitates, therefore, the use of transformations in an attempt to normalise the distribution of the variables (square root and logarithmic transformations are among the most commonly used, see Basáñez *et al.*, 1994) for application of least squares regression methods using the means in groups of flies. Maximum likelihood estimation with over-dispersed distribution of residuals is a powerful method that permits use of individual fly data (Subramanian *et al.*, 1998). One of the over-dispersed distributions most frequently used is the negative binomial, as it has proven to fit satisfactorily the number of *Onchocerca* larvae per fly among a range of vector species (Cheke *et al.*, 1982 and Renz, 1987 for *S. damnosum* s.l.; Demanou *et al.*, 2003 for *S. squamosum* B; Wetten *et al.*, 2007 for *S. exiguum* s.l., and Grillet *et al.*, 2008 for *S. oyapockense* s.l. and *S. incrustatum*). For measures of central tendency, authors tend to report the arithmetic mean, the median, the geometric mean of Williams (Williams, 1937) and if the variance is stabilised, to perform parametric statistics using log-transformed data and report confidence intervals of geometric means (Kirkwood and Sterne, 2003). The issue of which constant to add to parasite counts before taking their logarithm so that uninfected flies can be included in the analyses depends on the frequency of very low counts (when adding 1

TABLE 11.2 Possible functional forms to describe the relationships between two parasite developmental stages in the *Onchocerca-Simulium* interaction

Functional form	Behaviour	Shape of the relationship between y and x[a]	Parasite yield
Linear	Proportionality (no density dependence)		
Non-linear, saturating	Limitation (negative density dependence)		
Non-linear, sigmoid	Initial facilitation and subsequent limitation (positive and negative density dependence)		

Notes:[a] y represents density of the output variable and x density of the input variable in arbitrary units. Examples of input variables are: microfilariae in the skin (microfilariae per mg or per snip), or microfilariae ingested per fly; examples of output variables are successful microfilariae or L3 larvae per fly (adapted from Sinden *et al.*, 2007).

would be inappropriate), and has been discussed by Anscombe (1948) and Soumbey-Alley *et al.* (2004), among others.

Since the explanatory variables when studying *Onchocerca–Simulium* interactions tend to be random variables themselves (e.g., microfilaridermia per milligram of skin or per skin snip; number of microfilariae ingested per fly, etc.) and not truly independent variables, the problem of accounting for measurement error in the explanatory variable is commonly encountered. Measurement error may arise, among other causes, because of the fact we are essentially sampling from a distribution, and because there may be observer's error and inter-observer variation. The presence of measurement error may attenuate relationships, making them more strongly non-linear (accentuating limitation) than they would otherwise be if we had knowledge of the true value of the explanatory variable in question (Carroll *et al.*, 1995). The papers by Basáñez *et al.* (1994), Demanou *et al.* (2003), Soumbey-Alley *et al.* (2004) and Wetten *et al.* (2007) describe various approaches to the problem of measurement error, with the paper by Soumbey-Alley and co-workers (2004) focusing on the method of *instrumental variables*, and that of Demanou and co-workers (2003) on the method of estimating the *reliability ratio*.

The negative binomial distribution (NBD) also provides a useful method for relating the proportion of individuals infected in the population sample (be this of flies or humans) to the mean parasite load via an over-dispersion parameter (best known in the parasitological literature as the k parameter), which may itself be a function of the mean intensity of infection. Such a relationship has been used to describe the proportion of flies with ingested microfilariae that have been fed on given microfilaridermias as well as the proportion of infective flies resulting from such feeds, allowing for inter-specific, inter-cytoform, and intra-specific comparisons of vector competence for varying parasite densities (Basáñez *et al.*, 1994, 1995; Demanou *et al.*, 2003; Grillet *et al.*, 2008; Wetten *et al.*, 2007).

11.2.2. Mathematical models of *Onchocerca–Simulium* population biology and implications for the control of human onchocerciasis

The influence of different *Onchocerca–Simulium* combinations on the population dynamics and population genetics of the parasite has been investigated using a range of deterministic mathematical models. Systems of ordinary (Basáñez and Ricárdez-Esquinca, 2001; Basáñez *et al.*, 2007) and partial (Filipe *et al.*, 2005) differential equations have been developed to describe mathematically the life cycle of *O. volvulus* within the human host and different *Simulium* species. Churcher *et al.* (2005) have explored

the effect of parasite over-dispersion on the introduction and persistence of the infection in models for West African savannah *O. volvulus-S. damnosum* s.s. Where possible the models have been calibrated using parameters estimated by applying the statistical methods summarised in the previous section. Parameters not measured from experimental studies for ethical reasons (e.g., parasite establishment rates within humans) were estimated by fitting models to data relating exposure to blackfly bites with endemic microfilarial load from a range of geographical locations (Basáñez *et al.*, 2002). These deterministic models have been extended to incorporate genetic heterogeneity within the parasite population in order to investigate how the parasite may evolve under various selective pressures, specifically those exerted by widespread use of the microfilaricidal drug ivermectin (Churcher and Basáñez, 2008a,b).

11.3. RESULTS

11.3.1. Relationship between the availability of microfilariae in the skin and microfilarial intake by the fly

In some species a proportional (e.g., *S. ochraceum* s.l. and *S. guianense* s.l.) or nearly proportional (*S. damnosum* s.l.) relationship between the numbers of microfilariae ingested per fly and the numbers per milligram of skin has been reported (Basáñez *et al.*, 1994), whereas in others (e.g., *S. oyapockense* s.l. and *S. incrustatum*), microfilarial intake is negatively density dependent (Grillet *et al.*, 2008) (Fig. 11.3A–D). Demanou *et al.* (2003) compared the intake of various species of the *S. damnosum* complex and concluded that this was, in part, species specific and generally higher among savannah species (*S. damnosum* s.s./*S. sirbanum*) than forest (*S. squamosum/S. yahense*) or forest-savannah mosaic (*S. soubrense/S. sanctipauli*) species given similar skin burdens. There were also differences within species (*S. squamosum* B exhibiting intakes closer to those of savannah flies than *S. squamosum* A or C). These authors argued that in addition to simuliid-specific factors, differences in intakes could also be related to differences in the depth of dermal microfilarial distribution between parasite strains (Bain *et al.*, 1986; Vuong *et al.*, 1988), or the severity of skin disease (lichenification) associated with heavy microfilaridermia, particularly in forest *Onchocerca–Simulium* combinations (Duke, 1962). In contrast to the means of microfilarial intake varying markedly among simuliid species, the prevalence versus intensity relationships of flies with ingested microfilariae show greater similarity among species (Basáñez *et al.*, 1994; Demanou *et al.*, 2003; Grillet *et al.*, 2008), with the proportion of flies having ingested parasites increasing rapidly with mean microfilarial load or intake reaching nearly 100% for high
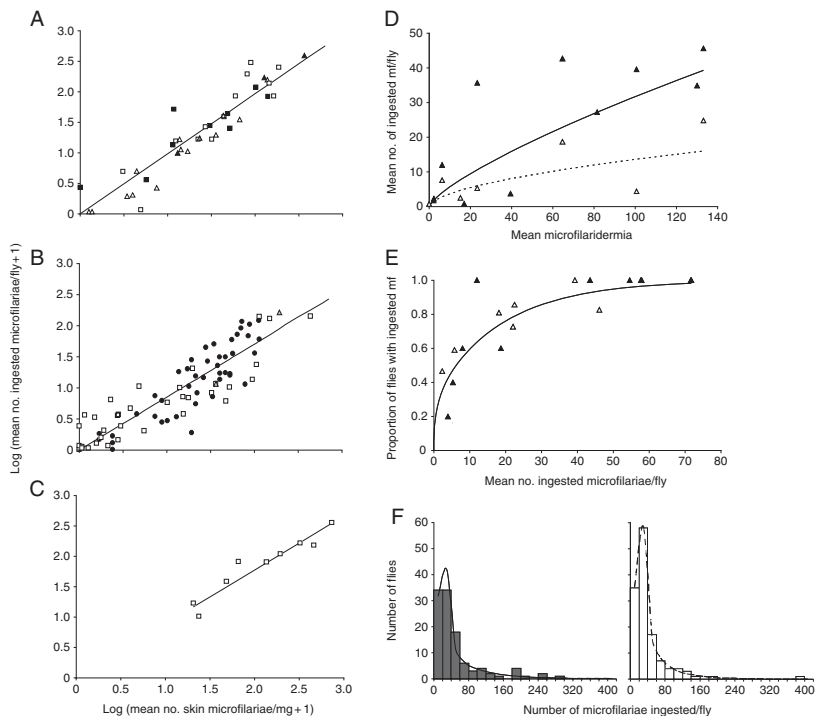
FIGURE 11.3   Ingestion of *O. volvulus* microfilariae by a range of simuliid vectors. The relationship between the number of microfilariae in the skin and the number of microfilariae ingested per fly in (A) *S. ochraceum* s.l. from Guatemala and Mexico; (B) *S. damnosum* s.l. from West African savannah; (C) *S. guianense* s.l., and (D) *S. oyapockense* s.l. (closed triangles) and *S. incrustatum* (open triangles) from the Amazonian focus. (E) The proportion of flies that ingested microfilariae as a function of mean microfilarial intake (markers as in (D)) with fitted function deriving from the negative binomial distribution of ingested microfilariae per fly that is depicted in (F). In (F) dark bars are for *S. oyapockense* s.l. ($k = 0.25$) and white bars for *S. incrustatum* ($k = 0.31$). In (A) open squares are from Campbell *et al.* (1980), closed squares from Collins *et al.* (1977), closed triangles from De Leon and Duke (1966) (Guatemala), and open triangles represent data obtained by Mario Alberto Rodríguez, Concepción Guadalupe Flores-Díaz, and Marco Alecio Sandoval (Mexico) for *S. ochraceum* s.l.; in (B) open squares are from Boussinesq (1991), closed circles are from data presented in Basáñez *et al.* (1995) and Soumbey-Alley *et al.* (2004), and grey triangles are from Philippon (1977) for savannah *S. damnosum* s.l.; (C) open squares are from Basáñez *et al.* (1994) for *S. guianense* s.l. in the Amazonian focus; (D–F) are redrawn from data presented by Grillet *et al.* (2008).

microfilaridermias (Fig. 11.3E). In those species studied, the number of microfilariae in the blood meal among flies fed on microfilarial carriers followed an over-dispersed distribution empirically well described by the NBD (Fig. 11.3F).

11.3.2. The survival of ingested microfilariae and that of the vectors

The proportion of microfilariae damaged by the cibarial armature has been found to decrease with microfilarial intake and to be species specific; Fig. 11.4 illustrates the differences in the proportion of ingested micro-filariae left unscathed between *S. ochraceum* s.l. (ranging from 0 for low intakes to 0.6 for high intakes; Fig. 11.4A), *S. oyapockense* s.l. (from 0 to 0.4; Fig. 11.4B), and *S. incrustatum* (from 0 to 0.8; Fig. 11.4C).

Basáñez *et al*. (1996) estimated that the life expectancy and, particu-larly, the infective life expectancy of groups of simuliids fed on microfi-larial carriers and kept in captivity was both fly's age dependent and dependent on mean microfilarial intake. The decrease of life expectancy with increasing ingested burden was more pronounced in blackflies lacking a well-developed cibarial armature than in 'armed' vectors (Fig. 11.5A and 11.5B). Survivorship of wild-caught flies with sharp armatures depends, however, not only on microfilarial load but also on the age-structure of the biting fly population (Fig. 11.5C and 11.5D).

11.3.3. The establishment and development of *Onchocerca* within the thorax of *Simulium*

11.3.3.1. Passage through the peritrophic matrix

The passage of microfilariae through the peritrophic matrix towards the ecto-peritrophic space, and through the abdominal epithelium into the haemocoel and towards the thoracic muscles has been studied both in terms of the dynamics of parasite numbers in the various fly compart-ments with time post-feeding (Lewis, 1953 and Laurence, 1966 for *S. damnosum* s.l.), and in terms of density-dependent relationships between parasite numbers in the consecutive compartments at given times after the blood meal (Philippon and Bain, 1972 and Soumbey-Alley *et al*., 2004 for *S. damnosum* s.l.; Bain *et al*., 1974 for *S. ochraceum* s.l.). Demanou *et al*. (2003) investigated both aspects in *S. squamosum* B. Microfilariae can be detected in the ecto-peritrophic space as soon as 2–10 min and in the thorax as soon as 20–30 min PE, the numbers in the thorax increasing according to an S-shape curve, and those in the blood meal steadily decreasing (Laurence, 1966). The numbers of 'successful' microfilariae (those escaping imprisonment by the PM, including ecto-peritrophic and haemocoelic microfilariae) plateau between 6 and 8 h PE (Demanou *et al*., 2003), and by 10–12 h PE most of the migration to the thorax has taken place. Jerwood *et al*. (1984) modelled the migration of *O. volvulus* microfilariae within *S. damnosum* s.l. using a simple compart-mental process with different transition rates between compartments (blood meal, abdominal haemocoel, thorax), and estimated an average
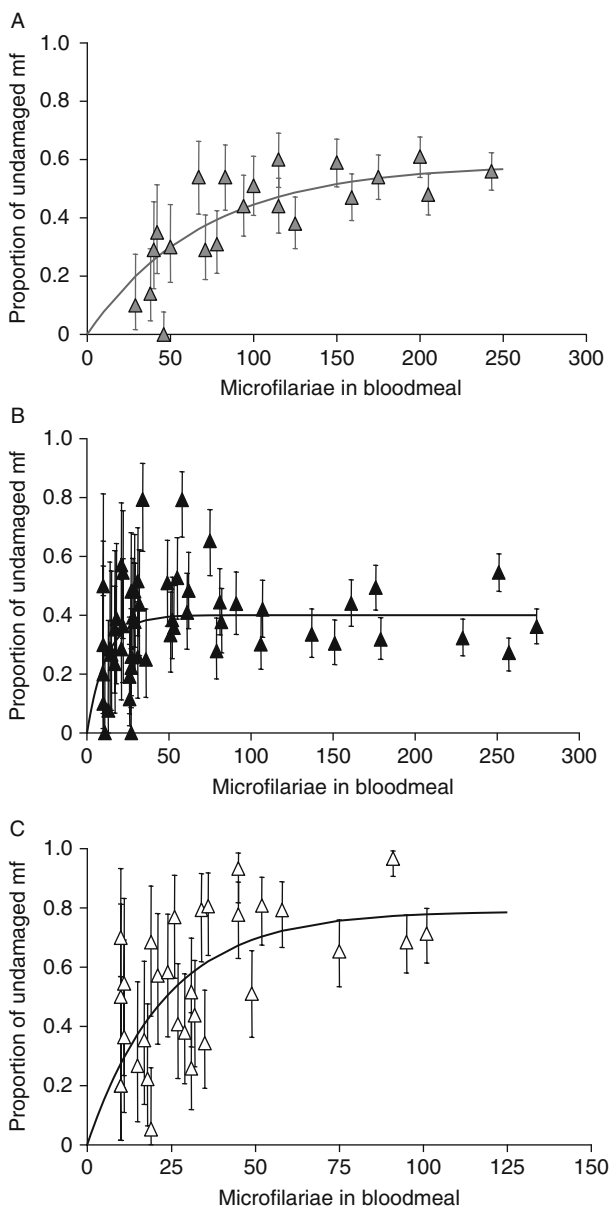
FIGURE 11.4   Proportion of ingested microfilariae left unscathed by the cibarial arma-ture. (A) *S. ochraceum* s.l. from Guatemala (data represented by grey triangles from Bain *et al.,* 1974), (B) *S. oyapockense* s.l. and (C) *S. incrustatum* from the Amazonian focus (data represented, respectively, by black and white triangles, from Grillet *et al.,* 2008). A saturating function described by Basáñez *et al.* (1995) was fitted to the data by maximum likelihood, indicating that the number of microfilariae (mf) lacerated at this
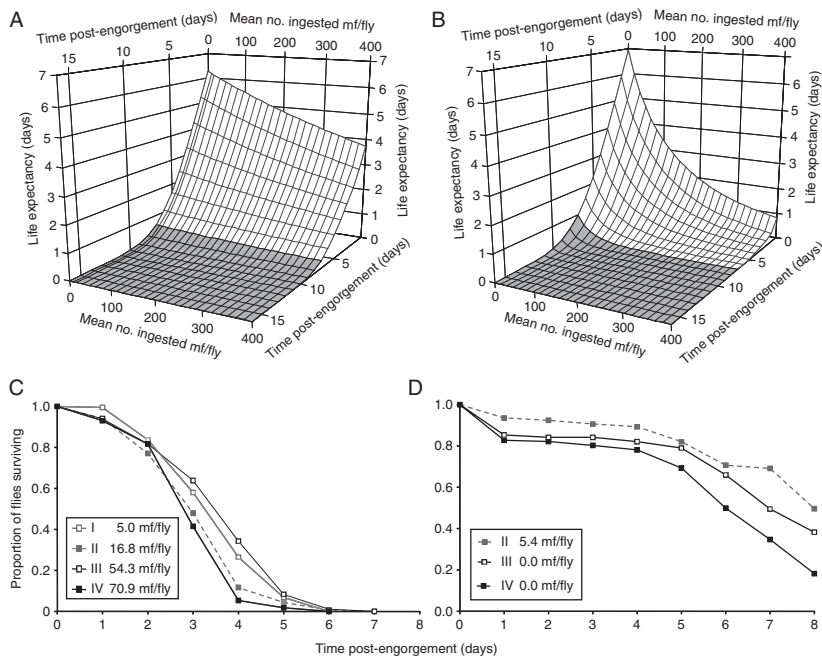
FIGURE 11.5   Effect of *O. volvulus* on the survival of *Simulium.* The (modelled) life expectancy (in days) as a function of both time post-engorgement (PE) (measuring fly's age) and mean microfilarial (mf) intake (measuring parasite load) in (A) simuliids with a well-developed cibarial armature (*S. ochraceum* s.l. from Guatemala), and (B) simuliids with a poorly developed armature (*S. damnosum* s.l. from West Africa and *S. guianense* s.l. from the Amazonian focus). The grey-shaded area represents the expectation of infective life assuming an extrinsic incubation period of 7 days (see Fig. 11.6B). Life expectancy and infective life expectancy decrease faster with increasing microfilarial load in simuliids not protected from parasite-induced damage by a well-developed cibarial armature. In both groups of flies the mortality rate increases with age (original analyses in Basáñez *et al.,* 1996). The proportion of surviving *S. oyapockense* s.l. with time PE is plotted for flies fed on microfilarial carriers before (C), and 6 months after ivermectin treatment (D), in the Venezuelan part of the Amazonian focus. When fed on the same subjects (I–IV), fly survivorship is better after microfilaridermia has been substantially reduced, but differences in the age-structure of the fly population 6 months apart may have also played a part (compare survivorship in both groups for ~5 mf/fly).

stage of the Onchocerca–Simulium interaction within armed vectors is both density dependent and species specific, with the percent of undamaged parasites ranging from 0% to 60% in *S. ochraceum*, 0% to 40% in *S. oyapockense* and 0% to 80% in *S. incrustatum.* Error bars are exact 95% confidence intervals. The various scales in the x-axis reflect species-specific differences in microfilarial intakes.

duration of 1.6 h in the blood meal, and of 3.1 h in the haemocoel, confirming that those parasites that migrate out of the PM early are the most likely to reach the thorax and proceed towards further development. Fig. 11.6A depicts the observed and predicted proportion of microfilariae in each compartment with time PE.

11.3.3.2. Successful microfilariae and L3 larvae versus available microfilariae per fly

When plotting the numbers and/or proportions of 'successful' microfilariae (those that have migrated out of the blood meal), or the numbers of L3 larvae that develop against microfilarial load in the skin area upon which groups of flies have been fed or against mean microfilarial intake, three distinct patterns emerge (Table 11.1): 1) African savannah flies of the *S. damnosum* complex (Fig. 11.7A–C) and the remaining Latin American simuliid vectors with poorly developed cibarial armatures exhibit limitation (*S. exiguum* s.l. (Fig. 11.8C and 11.D), *S. metallicum* s.l. (Fig. 11.8E), *S. guianense* s.l. (Fig. 11.8F)); 2) vectors with well-developed cibarial armatures exhibit initial facilitation (*S. ochraceum* s.l. (Fig. 11.8A and 11.B), *S. oyapockense* s.l. and *S. incrustatum* (Fig. 11.8G)), and 3) African forest and forest-savannah mosaic flies exhibit proportionality (*S. leonense* (Fig. 11.7D), *S. squamosum* B (Fig. 11.7E, F)), with numbers of successful microfilariae 10-fold higher than in savannah flies (however, how exactly this impacts L3 yield or vector survival needs further investigation). In forest foci, larval loads per naturally infected fly are also higher and the annual transmission potential (ATP, the yearly number of L3 that a person would potentially receive if maximally exposed to blackfly bites) can easily exceed levels that would be intolerable in the savannah because of their association with high blindness prevalence (Duke, 1968). The 'milder' forest strain might also be less virulent to *Simulium*, or may elicit a weaker immune response within the vectors.

Bain *et al.* (1976) proposed that parasite density-dependent changes in the thickness and rate of formation of the PM may explain the limitation observed in the number of ingested microfilariae that gain access to the thoracic muscles in savannah species of *S. damnosum* s.l. Other possible explanations include the activation of insect defences such as lectin-like molecules that interfere with microfilarial migration in filarial-culicid systems by acting at the level of the mid-gut epithelium (Phiri and Ham, 1990). At the level of the blackfly's haemocoel, Hagen and Kläger (2001) observed rapid (and species-specific) killing of *Onchocerca* microfilariae that was mediated by haemocytes and involved increased apoptosis levels in the microfilariae. Humoral (haemolymph) responses identified in simuliids include chiefly two types of inducible immune molecules, the serine proteases and the carbohydrate-binding lectins (Hagen *et al.*, 1995, 1997a), with prophenoloxidase also being up-regulated in *Onchocerca*-infected
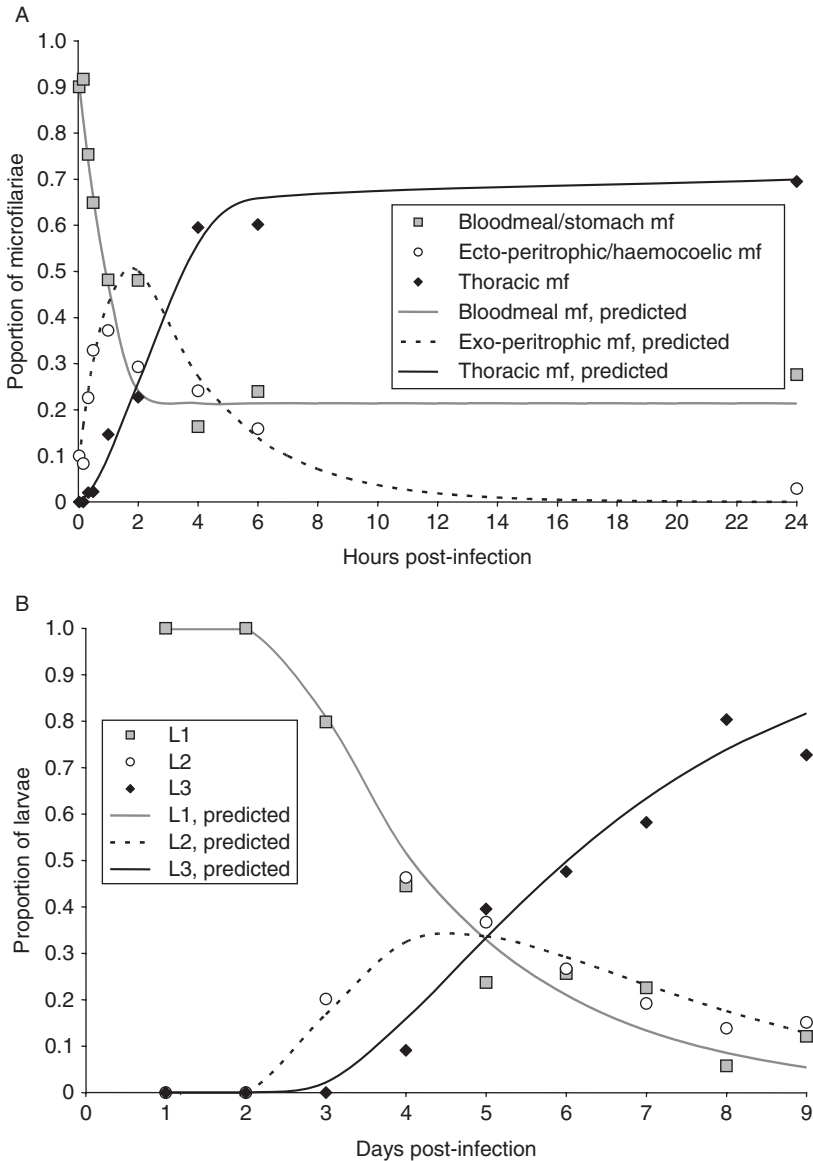
FIGURE 11.6 Compartmental models. (A) Migration of *O. volvulus* microfilariae from the blood meal to the thoracic muscles in *S. damnosum* s.l. in West Africa (data from Laurence, 1966; model by Jerwood *et al.*, 1984), and (B) development of *O. volvulus* larvae from the L1 to the L3 stage within the thoracic muscles of *S. exiguum* s.l. in Ecuador (data collated by Wetten *et al.*, 2007). The average durations in each compartment (A) or developmental stage (B) are the reciprocal of the transition rates estimated by the models. Microfilariae (mf) leave the blood meal on average 1.6 h and the abdomen

Simulium damnosum s.l. (Hagen *et al.*, 1997b)—although melanisation of larvae does not take place. More recently, other types of inducible peptides with anti-bacterial and anti-parasitic activity have been identified in black-fly's haemolymph; similarities of the immune response kinetics between bacterial and filarial infections suggest that intracellular *Wolbachia* bacteria, released from microfilariae, could be responsible for the anti-bacterial response (Kläger *et al.*, 2002). Although it is tempting to suggest that these *Simulium* immune responses may be involved in the phenomenon of limitation, more research is needed to elucidate whether their strength against *Onchocerca* larvae (see also Ham, 1986; Ham *et al.*, 1990, 1994) correlates positively with the density of the inoculum, or with parasite strain. The West African forest strain has lower *Wolbachia* levels (Higazi *et al.*, 2005) and may elicit weaker anti-bacterial responses within simuliids, helping to explain the higher larval loads observed in *Onchocerca–Simulium* forest combinations.

Whereas in some *Onchocerca–Simulium* combinations (savannah species of the *S. damnosum* complex; *S. ochraceum* s.l., *S. guianense* s.l.) the numbers of exo-peritrophic (ecto-peritrophic plus haemocoelic) and thoracic microfilariae per fly are nearly 1:1 predictors of resultant numbers of L3 larvae (Basáñez *et al.*, 1995; Collins *et al.*, 1977; Soumbey-Alley *et al.*, 2004), in others thoracic establishment of early larval stages is not necessarily linked to competent vector status. In southern Venezuela, anthropophagic populations of *S. exiguum* s.l. outside endemic areas are able to ingest *O. volvulus* microfilariae and allow them to reach and penetrate the thoracic muscle fibres. However, larval development does not proceed beyond the L1 stage, failing at the first moulting, with internal parasite structures becoming vacuolated and disintegrated (Basáñez *et al.*, 2000). These *S. exiguum* s.l. populations are, therefore, refractory to *O. volvulus*. By contrast, *S. exiguum* s.l. is the only known vector in Colombia (Tidwell *et al.*, 1980), and the Cayapa cytoform of the *exiguum* complex is a very efficient vector in Ecuador (Shelley and Arzube, 1985), though the numbers of thoracic larvae that establish surpass the numbers of L3 larvae

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(ecto-peritrophic space and abdominal haemocoel) 3.1 h after being ingested. After 6 h PE there is no much change in the numbers of microfilariae reaching the thorax. Once in the thorax, larvae remain as L1 larvae for 4.8 days and as L2 larvae for 1.9 days on average; L3 larvae can be seen as early as 4–5 days in the thorax of S. exiguum as a result of natural larval progression. Models were fitted by multinomial maximum likelihood. In (A) grey squares and solid line represent, respectively, observed and modelled proportions of hourly total microfilariae that are in the blood meal (stomach), open circles and broken line correspond to abdominal microfilariae that have left the stomach, and black diamonds and solid line to microfilariae that have reached the thorax. In (B) the same markers and lines have been used to represent, respectively, data and model fits for the proportions of total daily larvae that are in the L1, L2, and L3 stages.
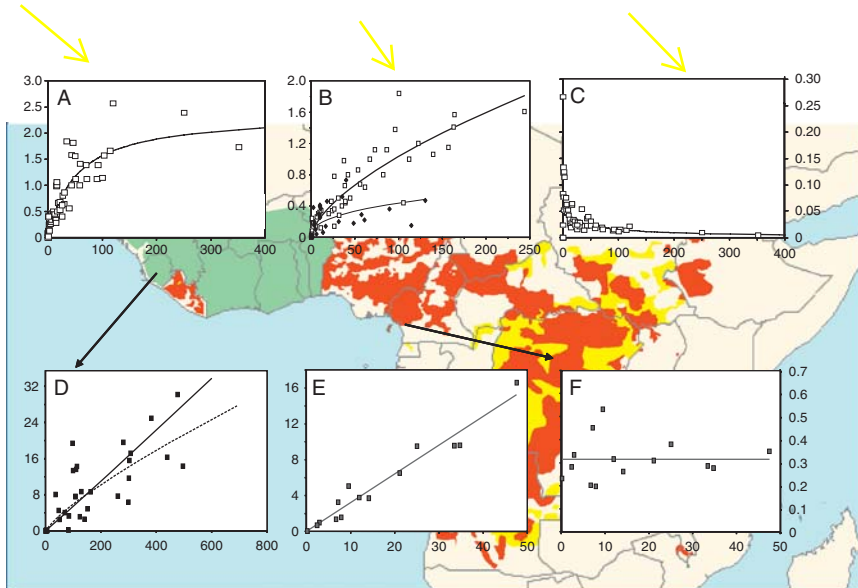
FIGURE 11.7 Relationships between successful and ingested or skin microfilariae in *S. damnosum* s.l. Upper panel (A–C) corresponds to Sudan-savannah species, and lower panel (D–F) to forest/forest-savannah mosaic species. In (A) the y-axis represents mean number of exo-peritrophic and thoracic microfilariae per fly in *S. damnosum* s.s./*S. sirbanum* (open squares) analysed together and the x-axis is mean microfilarial intake (data and saturating model as described in Basáñez *et al.,* 1995); in (B) *S. damnosum* s.s. (open squares) and *S. sirbanum* (closed diamonds) are analysed separately (data and model as described in Soumbey-Alley *et al.,* 2004) and the x-axis is mean microfilaridermia per skin snip. In (C), parasite yield (on the right y-axis) decreases monotonically with increasing microfilarial intake (x-axis), indicating limitation of microfilarial uptake in the Sudan-savannah species. (D) The relationship between exo-peritrophic microfilariae per fly (y-axis) and skin microfilariae per snip (x-axis) for *S. leonense* (forest-savannah mosaic, Sierra Leone), indicating a linearly proportional relationship between the two variables (the dotted non-linear fit is not statistically significantly better than the straight line), with an approximately constant microfilarial uptake of 4–5%; data and analysis (black squares and fitted lines) from Soumbey-Alley *et al.* (2004). (E, F) *S. squamosum* B, from the Sanaga valley in Cameroon (forest) exhibits proportionality, with (E) representing the number of hae-mocoelic microfilariae (y-axis) versus the number of ingested microfilariae (x-axis) per fly, and (F) the resultant (y-axis) parasite yield (an approximately constant 30% microfilarial uptake); data and analysis (grey squares and fitted lines) from Demanou *et al.* (2003).

produced (Collins *et al.,* 1995). This suggests the operation of yet unknown factors within the fly's thorax regulating parasite density, com-petition among larvae for space and resources, etc. In other cytoforms of the complex (Aguarico, Bucay and Quevedo) parasite yield has been shown to be significantly lower after adjusting for the limitation effect of negative density dependence (Wetten *et al.,* 2007). We have developed a simple compartmental model to estimate the transition rates and average
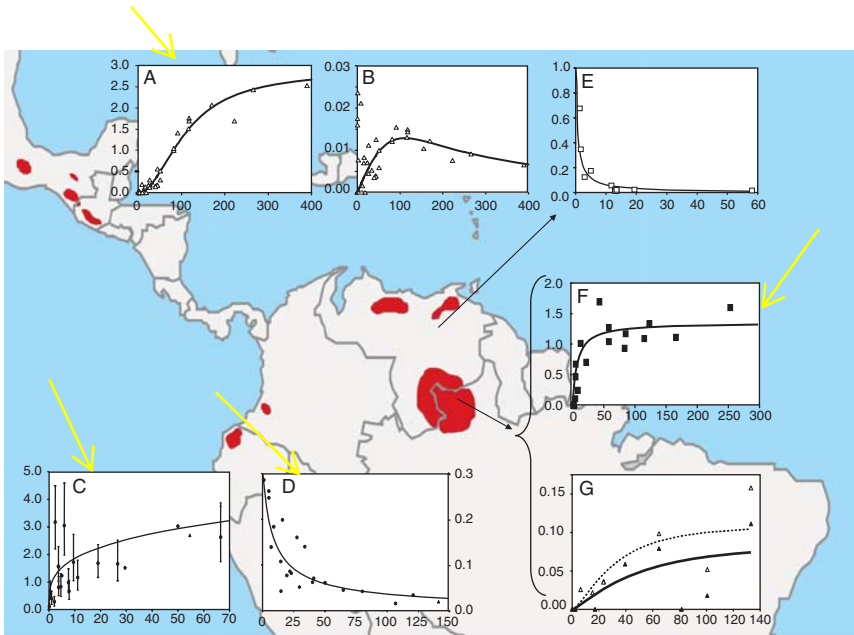
FIGURE 11.8   Relationships between successful and ingested or skin microfilariae in Latin American vector complexes. (A) and (B) represent, respectively, the number of successful microfilariae or L3 larvae per fly and the corresponding parasite yield (y-axes) versus mean microfilarial intake (x-axes) for *S. ochraceum* s.l. in Guatemala (data as grey open triangles collated by Basáñez *et al.,* 1995, with sigmoid grey line fit indicating initial facilitation. Parasite yield initially increases and subsequently decreases with mean number of microfilariae ingested per fly). (C) The number of L3 larvae per fly (left y-axis) and (D) parasite yield (right y-axis) versus microfilaridermia (per mg of skin, on the x-axes) for the Cayapa form of *S. exiguum* s.l. in Ecuador (data as black symbols collated by Wetten *et al.,* 2007, with circles from Collins *et al.,* 1995, diamond from Tidwell *et al.,* 1980, triangle from Shelley and Arzube, 1985, and non-linear black line fit indicating limitation). Parasite yield decreases monotonically with microfilarial density. (E) Parasite yield (y-axis) versus microfilaridermia (x-axis) in *S. metallicum* s.l. from northern Venezuela, data collated by Basáñez *et al.* (2000) demonstrating limitation. (F) The number of successful microfilariae or L3 larvae (y-axis) versus microfilarial intake (x-axis) for *S. guianense* s.l. from the Amazonian focus, with data (black squares) and saturating model fit (black line) presented by Basáñez *et al.* (1995). (G) The mean number of L3 per fly (y-axis) versus skin microfilarial load (x-axis) for *S. oyapockense* s.l. (black triangles for data and solid line for sigmoid model fit) and *S. incrustatum* (white triangles and broken line) from the Amazonian focus (Grillet *et al.,* 2008). The sigmoid fit in both these (armed) species suggests initial facilitation. (Background map prepared by Gaizka Ormaza.)

duration of each larval stage within the Cayapa form of *S. exiguum* s.l. (Fig. 11.6B). Allowing for an initial delay in the L1 compartment and variable transition rates, the mean duration of *O. volvulus* as an L1 larva is 4.8 days and of an L2 larva is 1.9 days.

In *S. metallicum* s.l. the PM is thin (Lewis and Garnham, 1959) and its rate of formation slow (Omar and Garms, 1977), favouring migration of microfilariae out of the blood meal, and in northern Venezuela, populations of *S. metallicum* s.l. from endemic areas allow thoracic establishment (subject to density-dependent limitation, Fig. 11.8E). Yet, larval development tends to be asynchronic, substantially reducing parasite yield (Basáñez *et al.*, 2000; Grillet *et al.*, 1994).

11.3.4. Local adaptation of *Onchocerca–Simulium* and its consequences for the spread of human onchocerciasis outside currently endemic areas

The notion of well-adapted *Onchocerca–Simulium* complexes (Duke *et al.*, 1966) was based on the observation that, in Cameroon, microfilariae of West African forest parasites developed efficiently in *S. damnosum* from West African forest but poorly or not at all in Sudan-savannah flies. Conversely, the success of Sudan-savannah parasites was high when developing within savannah *S. damnosum* but significantly reduced within forest flies (see also Philippon, 1977). Analysis of a tandemly repeated DNA sequence family present in the genome of *O. volvulus* (O-150) confirmed that West African rainforest and savannah parasite populations are significantly different and that some barrier preventing genetic exchange between these two populations must have developed, the existence of vector–parasite complexes offering a possible explanation (Zimmerman *et al.*, 1994). However, the situation is likely to be more complex, as there was better development of the forest parasite strain in Guinea-savannah and forest-savannah mosaic-dwelling simuliids (Duke *et al.*, 1966). Changes in the epidemiological landscape of West Africa in the last two to three decades, partly due to the Onchocerciasis Control Programme (OCP), may have disrupted such complexes (Toé *et al.*, 1997). Interestingly, non-significant differences were found between Guatemalan and North Venezuelan *O. volvulus* populations in their ability to infect local simuliids (*S. ochraceum*, *S. metallicum*, *S. callidum* and *S. haematopotum* in Guatemala, and *S. metallicum* in Venezuela) (Duke, 1970; Takaoka *et al.*, 1986a). This was interpreted as close genetic proximity between these two parasite populations (Takaoka *et al.*, 1986b). Interestingly, West African forest parasites developed somewhat better in Guatemalan simuliids than Sudan-savannah parasites (De Leon and Duke, 1966). This may be in contrast with reported results that American populations of *O. volvulus* are more closely related to those of West African savannah than forest (Zimmerman *et al.*, 1994), or may be due to strain-specific antigenic stimulation of the *Simulium*'s immune system as mentioned above.

In an evolutionary context, the hypothesis that host–parasite interactions are expected to result in geographical patterns of adaptation in which parasites are better able to infect their local host populations (Lively and Jokela, 1996), has been confirmed by experimental infection studies across a wide range of host–parasite systems (Failloux *et al.*, 1995; Lively, 1989; Parker, 1985). Laurence and Pester (1967) described the relatively rapid adaptation of the filarial worm *Brugia patei* to a new mosquito host, *Aedes togoi*, in the laboratory and discussed the implications of this finding in explaining present distribution of filariases.

If mutually compatible *Onchocerca–Simulium* complexes exist within each main endemic area, the question arises as to what is the true potential for onchocerciasis to spread towards new regions where infected carriers may settle and high densities of anthropophagic simuliids occur. Evidence of locally acquired onchocercal infections, or potential for this to take place outside original endemic areas has been documented in Brazil and Ecuador (Charalambous *et al.*, 1997; Guderian and Shelley, 1992; Maia-Herzog *et al.*, 1999). In Venezuela, Basáñez *et al.* (2000) investigated the compatibility between sympatric and allopatric combinations of *O. volvulus-Simulium* in the northern onchocerciasis focus (where flies of *S. metallicum* cytospecies E were fed on microfilarial carriers from the northern and Amazonian foci), and in a densely populated locality of Amazonas outside the main Amazonian focus (where *S. oyapockense* s.l. and *S. exiguum* s.l. were fed on the aforementioned microfilarial carriers). For the homologous northern *O. volvulus-S. metallicum* combination, parasite yield was 45% in contrast to 1% for the heterologous, Amazonian *O. volvulus-S. metallicum* infection. This was significantly lower than the parasite yield (4–10%) that would have been expected in the sympatric combination after allowing for density-dependent limitation of L3 output in *S. metallicum*. The anthropophagic population of *S. exiguum* s.l. from southern Venezuela allowed no larval development beyond the L1 stage of either northern or Amazonian parasites (see Section 11.3.3.2). The parasite yield of Amazonian *O. volvulus* in *S. oyapockense* s.l. biting humans in the capital of the Amazonas State was about 1%, in agreement with figures ranging from 0.02% to 1.23% recorded for the sympatric combination within the Amazonian focus (Grillet *et al.*, 2008). By contrast, no L3 development of the northern parasite was observed in southern *S. oyapockense*. These results, together with considerations of typical microfilarial loads in humans in the northern and Amazonian foci, poorly developed (*S. metallicum*) or well-developed (*S. oyapockense*) cibaria in the blackflies (Shelley *et al.*, 1987), parasite-induced vector mortality (high in *S. metallicum*; Omar and Garms, 1977), and fly biting rates (of the order of thousands per person per day for *S. oyapockense* s.l. in the Amazonian lowlands; Grillet *et al.*, 2001), suggest a lower potential for onchocerciasis to spread between the northern and Amazonian endemic areas of

Venezuela than that between Amazonian hyperendemic, untreated locations and settlements outside this focus with high densities of anthropophagic *S. oyapockense* s.l. Table 11.3 presents a (non-exhaustive) summary of experimental infections conducted to study the cross-compatibility of *Onchocerca–Simulium* combinations in African and Latin American endemic areas.

11.4. IMPLICATIONS FOR OUR UNDERSTANDING OF THE POPULATION AND EVOLUTIONARY BIOLOGY OF *O. VOLVULUS* AND THE CONTROL OF HUMAN ONCHOCERCIASIS

In this section we use mathematical models to coalesce and interpret the results of the previous sections and discuss how in-depth knowledge of vector–parasite interactions informs current understanding of the transmission dynamics of the infection and helps identify optimal control strategies. Fig. 11.9 presents the life cycle of *O. volvulus*, illustrating at various points the density-dependent processes that may be operating. Churcher *et al.* (2005) have combined density dependence and over-dispersed parasite frequency distributions in savannah *Onchocerca–Simulium* complexes, and Churcher *et al.* (2006) have investigated the separate and combined effects of density dependence on rates of parasite re-infection after treatment.

11.4.1. Consequences of density dependence in *Onchocerca–Simulium* interactions for efforts to achieve local elimination of *O. volvulus*

The aim of the Onchocerciasis Elimination Program for the Americas (OEPA) is to eliminate the *O. volvulus* reservoir in the Americas and not just the public health burden (Richards *et al.*, 2001). There are also focal areas within Africa where parasite elimination is deemed possible. The feasibility of this goal may well depend on the particular vector complex or complexes that are present in the areas targeted for elimination, particularly where programme objectives rely almost entirely on reducing microfilaridermia by mass administration of ivermectin. In principle, local parasite elimination can be achieved by lowering parasite density to such an extent that: (1) the remaining adult male and female worms do not inhabit the same host or females cannot be fertilised, thus not producing microfilariae (interrupting transmission from humans to vectors), or (2) most, if not all, of the few microfilariae ingested by armed simuliids (in areas where these prevail) are damaged, thus not proceeding towards

TABLE 11.3 Summary of cross-experimental infections to assess local adaptation of *Onchocerca-Simulium* complexes

Country (Type)[a]	*O. volvulus-Simulium* combination	Subject (Locality)	Microfilariae per fly	L3 larvae per fly	Parasite yield	Reference
Cameroon (S)	West Africa Forest	I (Bolo & Sanaga)	3.74–11.0	2.25–5.12	0.47–0.86	Duke *et al.*, 1966
	O. volvulus	II (Bolo)	0.94–8.60	0.66–5.60	0.65–0.86	
	& *Simulium*[b]					
Bioko (S)	W. A. Forest *O. volvulus*	I (Tiburones)	3.61	1.61	0.45	Duke *et al.*, 1966
	& *Simulium*[c]					
Sierra Leone (S)	W. A. Forest *O. volvulus*	I (Magburaka)	5.93	3.74	0.63	Duke *et al.*, 1966
	& *Simulium*[d]					
Cameroon Burkina Faso (S)	Sudan savannah *O. volvulus*	III (Mayo Boki & Volta Blanche)	1.10–4.05	0.40–1.88	0.36–0.47	Duke *et al.*, 1966
	& *Simulium*[e]	IV (M. Boki)	16.5	2.53	0.15	
Cameroon Burkina Faso (A)	W. A. Forest *O. volvulus-*	I (M. Boki & Grand Capitaine)	6.38–7.77	0–0.10	0–0.01	Duke *et al.*, 1966
	S. savannah *Simulium*	I (V. Blanche)	15.1	0	0	
		II (M. Boki)	2.18	0.19	0.09	
Cameroon (A)	S. savannah *O. volvulus-*	III (Bolo)	1.0–3.87	0–0.09	0–0.02	Duke *et al.*, 1966
	W. A. Forest *Simulium*	IV (Bolo)	18.5	0.14	0.01	
Sierra Leone (A)	S. savannah *O. volvulus-*	III (Magburaka)	1.47	0.13	0.09	Duke *et al.*, 1966
	W. A. Forest *Simulium*					
Guatemala (S)	Guatemalan	I	9.0–390	0.19–2.53	0.02–0.01	De Leon & Duke, 1966
	O. volvulus-	II	170	2.07	0.01	
	S. ochraceum					
Guatemala (S)	Guatemalan	I	6.0	0.15	0.03	De Leon & Duke, 1966
	O. volvulus-	I	190	Intake fatal	0	
	S. metallicum	II	5.0	0.06	0.01	

Guatemala (A)	W. A. Forest *O. volvulus-S. ochraceum*	III	14.0	0.24	0.02	De Leon & Duke, 1966
Guatemala (A)	W. A. Forest *O. volvulus-S. metallicum*	III	16.0	0.05	0.003	De Leon & Duke, 1966
Guatemala (A)	S. savannah *O. volvulus-S. ochraceum*	IV	1.4	0	0	De Leon & Duke, 1966
Guatemala (A)	S. savannah *O. volvulus-S. metallicum*	IV	0.9	0	0	De Leon & Duke, 1966
Northern Venezuela (S)	Venezuelan *O. volvulus-S. metallicum*	VIII (Altamira)	2.78	0.58	0.21	Duke, 1970
		IX (Altamira)	9.85	0.86	0.09	
		X (Altamira)	19.9	0.66	0.03	
N. Venezuela (S)	Venezuelan *O. volvulus-S. exiguum*	VIII (El Loro)	1.63	0.09	0.06	Duke, 1970
		X (El Loro)	14.9	0.04	0.003	
N. Venezuela (A)	W. A. Forest *O. volvulus-S. metallicum*	VI (Altamira, Carabobo)	4.66	0	0	Duke, 1970
N. Venezuela (A)	S. savannah *O. volvulus-S. metallicum*	VII (Altamira, Carabobo)	24.0	0.01	0.0004	Duke, 1970
N. Venezuela (A)	W. A. Forest *O. volvulus-S. exiguum*	VI (El Loro, Aragua)	1.55	0	0	Duke, 1970
N. Venezuela (A)	S. savannah *O. volvulus-S. exiguum*	VII (El Loro, Aragua)	2.14	0	0	Duke, 1970
Guatemala (S)	Guatemalan *O. volvulus-S. metallicum*	I (Chimaltenango)	0.80	0.05	0.06	Takaoka et *al.*, 1986a

Table 11.3 (*continued*)

Country (Type)[a]	*O. volvulus-Simulium* combination	Subject (Locality)	Microfilariae per fly	L3 larvae per fly	Parasite yield	Reference
Guatemala (A)	Venezuelan *O. volvulus-* Guatemalan *S. metallicum*	II (Chimaltenango)	3.00	0.40	0.13	Takaoka *et al.,* 1986a
N. Venezuela (S)	Venezuelan *O. volvulus-* *S. metallicum*	II (Rio Chiquito, Monagas)	7.31	0.47	0.06	Takaoka *et al.,* 1986b
N. Venezuela (A)	Guatemalan *O. volvulus-* Venezuelan *S. metallicum*	I (Rio Chiquito, Monagas)	1.95	0.08	0.04	Takaoka *et al.,* 1986b
N. Venezuela (S)	N. Venezuelan *O. volvulus-* *S. metallicum*	Ia (Carrasposo, Anzoátegui)	2.13	0.95	0.45	Basáñez *et al.,* 2000
N. Venezuela (A)	Amazonian *O. volvulus-* N. Venezuelan *S. metallicum*	IIb (Carrasposo, Anzoátegui)	10.9	0.11	0.01	Basáñez *et al.,* 2000

[a] (S) Sympatric (homologous) combination, (A) Allopatric (heterologous) combination;
[b] Possibly *S. squamosum* at Sanaga
[c] *S. yahense* Bioko form
[d] *S. leonense*
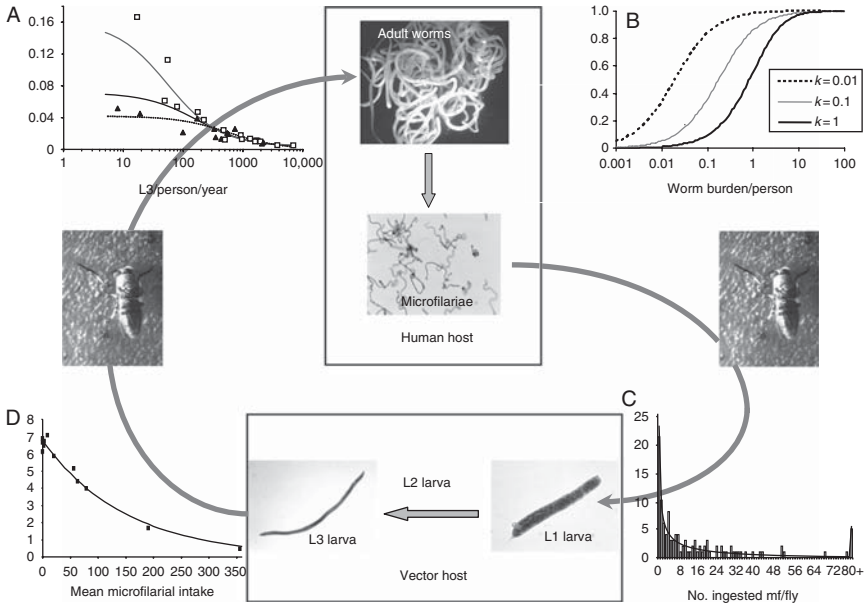[e] *S. damnosum s.s./S. sirbanum.*

FIGURE 11.9 Life cycle of *Onchocerca volvulus* and density-dependent processes.
(A) Transmission of L3 larvae via blackfly bites results in a per capita parasite establish-
ment rate that decreases with the rate of human exposure to infective larvae (negative
density dependence), with data and model fits redrawn from those presented by
Basáñez *et al.* (2002); open squares for West Africa and black triangles for Meso America,
with grey, black and dotted lines representing a range of plausible fits. Established
worms become adult males and females within subcutaneous nodules. (B) The proba-
bility that a female worm is fertilised (positive density dependence) increases both with
mean worm burden and degree of over-dispersion, with a decreasing value of parameter
k (from the negative binomial distribution, NBD) indicating stronger parasite clumping.
Given a mean worm burden, the mating probability increases faster for $k = 0.01$ (dotted
line), than for $k = 0.1$ (grey line) or $k = 1$ (solid black line). Fertilised females produce
microfilariae (mf) that migrate to the skin, being ingested with a blood meal (Fig. 11.2).
(C) The distribution of the number of microfilariae per fly is also clumped and well-fitted
by the NBD (adapted from Demanou *et al.* (2003) with $k = 0.4$). Microfilariae establish
within the thoracic muscles (Figs. 11.2 and 11.6A) with per capita rates of establishment
(uptake) that can decrease, initially increase or remain constant (i.e., can exhibit negative
(Figs. 11.7C, 11.8D and 11.8E), positive (Fig. 11.8B), or no density dependence (Fig. 11.7F).
(D) Life expectancy of the vector may be adversely affected by increasing microfilarial
intake, another form of negative density dependence (data jointly analysed for
S. damnosum and *S. guianense*, black markers and fitted line, from those presented by
Basáñez *et al.*, 1996). Photo credits: adult worms from the TDR Image Library (WHO/
TDR/OCP; image ID: 9303242); microfilariae emerged from incubated skin snips by
Carlos Botto, Amazonian Centre for Research and Control of Tropical Diseases (CAICET);
L1 and L3 larvae within *Simulium guianense* by María-Gloria Basáñez; biting female
blackfly (*S. guianense* from the Amazonian focus) by Carlos Ayesta, Faculty of Sciences,
Universidad Central de Venezuela.

larval development (interrupting transmission from vectors to humans). Therefore, both these transmission-blocking strategies rely on the operation of positive density dependence, the former affecting the probability that a female worm is mated (Fig. 11.9B), and the latter the probability that an ingested microfilaria develops into an L3 larva in simuliids with well-developed cibarial armatures and hence initial facilitation. The mating probability can in theory be estimated using information on mean worm burden per host, the ratio of male to female worms, the frequency distribution of worms per host and the sexual system of the parasites (May, 1977; May and Woolhouse, 1993), assumed to be polygamous in *O. volvulus* (Schulz-Key and Karam, 1986). Using recent estimates derived from fitting a mathematical model to microfilarial data from Guatemala, pre-ivermectin values for mean worm burden and corresponding (NBD) over-dispersion parameter were, respectively, of the order of 47 worms per person and k was 0.35 (Bottomley *et al.*, 2008). With these figures, and a balanced sex ratio, the initial mating probability is very close to 1 (all female worms would be mated). In practice the mating probability of a strongly polygamous and clumped parasite is likely to remain high during most of the control programme until the worm burden is substantially reduced, or the parasite sex ratio markedly altered (e.g., if male worms were significantly more sensitive to the drug), or female insemination rates were disrupted (Cupp and Cupp, 2005). By the same token, initial facilitation in armed blackflies is likely to have an impact only when the vector biting rate is very close to the threshold necessary to maintain endemic transmission; for biting rates well above this threshold in the absence of vector control, parasite densities below which transmission would be blocked (transmission breakpoints) will be extremely low. These concepts are illustrated by plotting the effective reproduction ratio (R_e) of *Onchocerca* against mean microfilaridermia (Fig. 11.10), although it must be stressed that the results presented here are only intended to provide qualitative insight rather than accurate quantitative predictions (as they are derived using a deterministic model and most likely overestimate the worms' mating probability). R_e is defined as the average number of adult female progeny produced during the reproductive life span of an adult female worm given a mean worm burden, and, therefore, is subject to density dependence (unlike the basic reproduction ratio, or R_0). The parasite intensity below which the operation of positive density dependence restricts population growth to such an extent that $R_e < 1$ (each female worm is unable to replace itself) is the breakpoint density (Macdonald, 1965). The effective reproduction ratio of *O. volvulus* in an area where *S. ochraceum* s.l. is the most important vector species is presented in Fig. 11.10A for different annual biting rates (ABR). Larger values of the vector biting rate reduce the breakpoint density, making *O. volvulus* harder to eliminate (Duerr *et al.*, 2005). Different
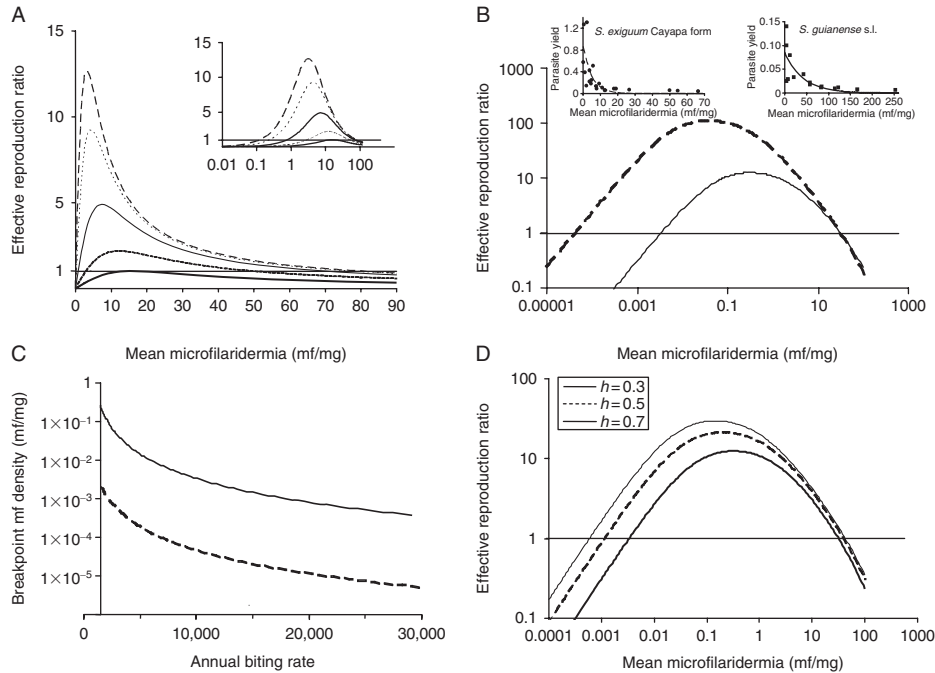
FIGURE 11.10 Transmission breakpoints in human onchocerciasis. The effective reproduction ratio (R_e, with density dependence) is plotted against mean microfilaridermia in (A), (B) and (D). A value of $R_e = 1$ indicates equilibrium (each female worm replaces itself, represented by the points where the curves cross the horizontal solid line), with endemic equilibria corresponding to those lying on the right-hand side of maximum R_e, and unstable equilibria (breakpoint densities) to those lying on the left-hand side. The lower the breakpoint densities are, the harder it will be to achieve local elimination of onchocerciasis. (A) R_e values for increasing annual biting rates (ABR = number of flies landing to bite as recorded in Mexico and Guatemala and collated by Basáñez *et al.*, 2002) for *S. ochraceum* s.l. (8,800 flies person^{-1} year^{-1}, thick solid,

Onchocerca–Simulium complexes have different types and severities of positive and negative density-dependent regulatory mechanisms which will alter the parasite breakpoint densities (Fig. 11.10B and 11.10C). Higher proportion of blood meals taken on humans by the local vector population will lower breakpoint microfilaridermia (Fig. 11.10D). However, care should be taken when interpreting breakpoint densities as some residual transmission may be maintained even when $R_e < 1$, and parasite burden may oscillate around the breakpoint density, taking many years to be truly eliminated (Gambhir and Michael, 2008). Increases in the vector population during this period, or seasonal changes in vector density (Grillet *et al.*, 2001; Vieira *et al.*, 2005) may allow the parasite to re-establish itself in the host population. In general, under the current strategy of mass distribution of ivermectin without additional measures of vector control, the risk of re-infection may be higher than previously assumed (Duerr *et al.*, 2006).

11.4.2. The evolution and spread of ivermectin resistance in *O. volvulus* populations

Current reliance of onchocerciasis control programmes on mass distribution of ivermectin, and reports of elevated microfilarial loads in treated individuals and populations (Awadzi *et al.*, 2004a,b; Osei-Atweneboana *et al.*, 2007) raise the prospect that drug resistance may become a public health concern (but see Cupp *et al.*, 2007; Mackenzie, 2007; and Remme *et al.*, 2007 for a debate as to whether the above mentioned observations

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bottom line (El Jardín); 33,127, thin dashed line (Nueva América); 92,585, thin solid line (Los Tarrales); 301,065, dotted line (Los Andes); 550,559, thick dashed line (Santa Isabel). The ABR at El Jardín is very close to the threshold biting rate assuming that all blood meals are taken on humans. As vector density increases, endemic microfilarial loads increase and transmission breakpoint densities decrease (see inset for details), highlighting that vector control could play a crucial role in aiding parasite elimination. (B) For two vector species with negative density dependence, and assuming equal endemic microfilarial load ($\sim$30 microfilariae (mf)/mg), the stronger limitation quantified for *S. exiguum* s.l. (upper left inset and dashed line) in comparison to that for *S. guianense* s.l. (upper right inset and solid line) reduces breakpoint microfilaridermia (ABR = 10,000; 1 in 3 blood meals are on human hosts). (C) The larger the endemic ABR value, the smaller the breakpoint microfilaridermia (solid line, *S. guianense*; dashed line, *S. exiguum*; anthropophagy as in (B)), highlighting once more the role that vector control could have in elimination efforts. (D) The more anthropophagic a vector population, the harder to achieve local elimination, with the proportion of blood meals taken on humans (*h*) equal to 0.3 (thick solid, bottom line), 0.5 (dashed line), and 0.7 (thin solid, top line) for *S. guianense* s.l. with ABR = 10,000. Predicted endemic microfilaridermia values range from $\sim$30 (*h* = 0.3) to $\sim$40 (*h* = 0.7) mf/mg.

may be due to causes other than drug resistance). If resistance alleles were present within populations of *O. volvulus* (it is expected that they would initially be rare), their rate of spread would be influenced by the density, competence and biting behaviour of the local simuliid vectors.

Predicting how drug resistance will spread through different *Onchocerca–Simulium* complexes requires a fuller understanding of the genetics of ivermectin resistance in human filariases, as well as of the processes (immunological or otherwise) that regulate parasite establishment within the human host and how these will be affected by chemotherapy. Some onchocerciasis mathematical models assume that L3 larvae provide the antigenic stimulus for protective immune responses and that immunological memory is relatively short, with the per capita rate of parasite establishment decreasing with increasing ATP (Fig. 11.9A) (Basáñez *et al.*, 2002; Duerr *et al.*, 2008). As mass drug administration progresses, microfilaridermia in treated hosts and overall transmission would initially decrease. Decreased ATP levels would result in increased parasite establishment rates, giving an advantage to any resistant parasites that may be present (whose microfilariae would have had a greater chance of reaching the L3 stage). As resistant parasites replace the drug-sensitive worm population, areas with higher vector biting rates and/or highly competent vectors will also recover higher ATPs, with higher net numbers of parasites and skin microfilariae. Therefore, drug resistance might become more evident in areas with higher vector biting rates and endemic transmission potentials in the absence of vector control (Fig. 11.11B). Other models have assumed facilitated parasite establishment mediated by the number of adult worms already present (Duerr *et al.*, 2003). Depending on whether or not repeated ivermectin treatment has macrofilaricidal effects (Cupp and Cupp, 2005), establishment rates of resistant parasites may be initially reduced as drug-sensitive parasites are affected. Ivermectin acts by both killing *O. volvulus* microfilariae (microfilaricidal effect) and inhibiting their production by female worms (embryostatic effect) for several weeks (Basáñez *et al.*, 2008). Therefore, repeated ivermectin treatment would result in rapid and substantial changes of putative resistance allele frequency in populations of skin microfilariae and L3 larvae in vectors (Fig. 11.11C).

Treatment would reduce the impact of negative density-dependent processes restricting parasite abundance in humans and vectors. The relaxation of these regulatory processes will increase the contribution of resistant parasites to the subsequent generation (Churcher and Basáñez, 2008a,b). Vectors that exert strong limitation on microfilarial uptake will tend to give resistance alleles greater selective advantage after chemotherapy. This is because most drug-sensitive parasites will be within those untreated hosts who have high microfilaridermia. Conversely, drug-resistant parasites will be harboured by treated hosts, whose initial

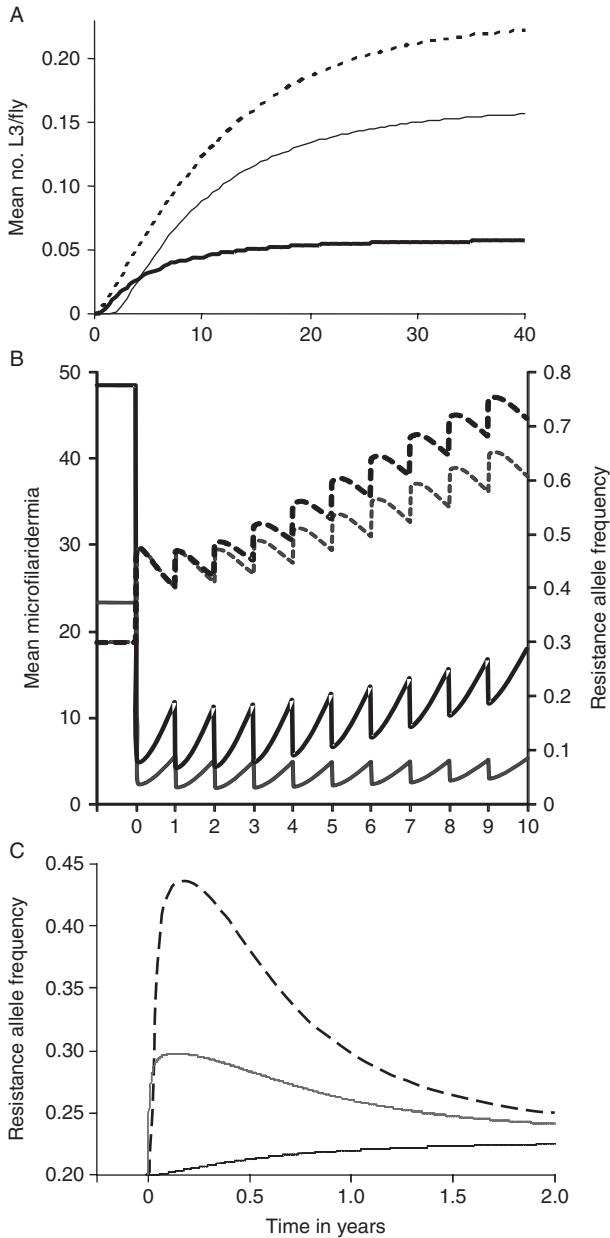**FIGURE 11.11**   Spread of anthelmintic resistance in *O. volvulus*. (A) Predicted mean infective larval load in Sudan-savannah *Onchocerca–Simulium* combinations by models that ignore (dotted line) or incorporate (solid lines) parasite frequency distribution plus density dependence in vectors and humans. The mean-based model overestimates infective larval load and does not capture appropriately initial dynamics at the time of

microfilaridermia will be lower, increasing parasite yield. Also, stronger limitation will lower transmission thresholds (Fig. 11.10B and 11.10C), making local elimination more difficult. Vectors with strong facilitation may, on the contrary, restrict the spread of drug-resistant parasites, particularly at low resistance allele frequencies.

The number of bites received by hosts may depend on their age and sex (Filipe *et al.*, 2005). A heterogeneous biting rate may cause hosts to acquire new infections at different rates, which could result in the resistance allele frequency varying between hosts (Churcher and Basáñez, 2008b). At low allele frequencies parasite genetic differentiation between hosts enhances the spread of recessive alleles, by increasing the proportion of offspring that will be homozygous. Under the assumption that ivermectin resistance is recessive, this will lead to a faster spread of resistant parasites (Schwab *et al.*, 2007). The genetics of ivermectin resistance in *O. volvulus*, however, is likely to be more complex and possibly polygenic.

Should drug resistance develop in one area of the control programme, it is likely to spread geographically relatively quickly. Blackflies are known to migrate long distances, both in search of suitable breeding sites and through wind-assisted movement (Boakye *et al.*, 1998). Results from the small number of genetic studies that have been undertaken in *O. volvulus* suggest that there is limited intra-specific structure

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introduction. The models with moderate ($k = 0.1$, thin solid line) and strong ($k = 0.01$, thick solid line) parasite aggregation predict a lower larval load and enhance the probability of introduction and persistence (redrawn from Churcher *et al.*, 2005). (B) The influence of vector biting rate on the detection (by increasing microfilaridermia, left-hand y-axis, solid lines) and spread (resistance allele frequency, right-hand y-axis, dashed lines) of ivermectin resistance as predicted by mathematical models that introduce genetic structure into *O. volvulus* populations with respect to drug susceptibility. The model (Churcher and Basáñez, 2008a) has been parameterised for *Onchocerca–Simulium* combinations from the Sudan-savannah bioclimatic zone. The annual biting rate is 15,000 (black lines) or 2,000 (grey lines). Ivermectin coverage is at 80% annually for 10 years. For illustrative purposes only, drug resistance is conferred by a single, autosomal recessive allele which protects the parasite from the microfilaricidal and embryostatic effects of ivermectin. Initial resistance allele frequency is 0.3. (C) The impact of ivermectin treatment on mean (within treated and untreated hosts) resistance allele frequency of different *O. volvulus* life-stages: adult worms within humans (solid black line); microfilariae in skin (grey line), and L3 within vectors (dashed line). The temporal dynamics are shown following a single round of ivermectin to 80% of the human population. Assumptions are as in (B) excepting initial resistance allele frequency which equals 0.2 (redrawn from Churcher and Basáñez, 2008a). Sampling infective larvae in vector populations may be an appropriate strategy to monitor changes in resistance allele frequency following chemotherapy.

(Morales-Hojas *et al.*, 2007). This would facilitate the flow of resistance alleles between different African populations.

11.5. DISCUSSION AND FUTURE RESEARCH DIRECTIONS

The centre for adaptive radiation of the genus *Onchocerca* (mainly parasites of ungulate mammals) is the African continent (Bain, 1981), with *O. volvulus* belonging to a small, highly specialised group that evolved from parasites of African savannah bovids (Krueger *et al.*, 2007). The African savannah and forest strains of *O. volvulus* are genetically distinct (Zimmerman *et al.*, 1994), transmitted on the whole by different simuliid species and correlate well, respectively, with severe and mild ocular disease (Higazi *et al.*, 2005; Zimmerman *et al.*, 1992). The greater compatibility between sympatric as opposed to allopatric parasite–vector combinations suggests the operation of local adaptation, which would provide indirect evidence of co-evolution (Woolhouse *et al.*, 2002). In practice, demonstrating co-evolution in *Onchocerca–Simulium* would involve testing for reciprocal adaptive genetic change, and documenting fitness benefits for either parasite or vector associated with the trait in question (Poulin, 1998). However, lack of animal models for *O. volvulus* and difficulties in colonising *Simulium* in the laboratory have hampered understanding of their formal genetics and hindered opportunities for tightly controlled experimental approaches.

It has been proposed that natural selection would favour parasites that can manipulate their vectors to enhance their transmission (Hurd, 2003). The factors that contribute to parasite transmission success are encapsulated in the formulation of its basic reproduction ratio, R_0. With respect to the *Onchocerca–Simulium* interactions, crucial factors are the amount of contact between vectors and humans (when parasites are transmitted to and from blackflies), the probability that an ingested microfilaria reaches the infective stage (vector competence), and the probability that infected flies survive completion of *O. volvulus* extrinsic incubation period and beyond (vector survivorship and infective life expectancy). Therefore, parasite strategies may include increasing the contact rate (to our knowledge, an area of research not much pursued in blackflies), enhancing the input of microfilariae or the output of L3 larvae, augmenting vector competence, and lengthening vector longevity. Vectors, in turn, may mount anti-parasitic defences or develop parasite-avoidance strategies, while the parasites may develop the ability to evade or suppress host defences (Hurd, 2003; Koella, 1999).

In Section 11.3.3.2 work confirming the operation of innate and acquired resistance to filarial infection in blackflies was summarised. As a possible counteracting mechanism, Kläger *et al.* (1999) found a

cysteine protease inhibitor, onchocystatin, which was present in female adults, skin microfilariae and excretory-secretory microfilarial products of the bovine parasite *O. ochengi* Bwangamoi (the closest relative of *O. volvulus*). Co-injection of onchocystatin and *O. ochengi* microfilariae into the thorax of surrogate host *Simulium ornatum* Meigen s.l. signifi-cantly enhanced parasite establishment rates within 24 h post-infection, suggesting a possible role of onchocystatin in the evasion by the parasite of the simuliid's immune response. Further research could focus on con-firming the role of onchocystatin in the evasion of immune responses by members of the *S. damnosum* complex, the natural vectors of *O. ochengi* (Omar *et al.*, 1979; Wahl *et al.*, 1998).

Another strategy for increasing parasite transmission success would be for the parasite to deplete vector's reproductive output. This would increase nutrient reserves available for the parasite while increasing vec-tor longevity, as decreased oviposition rates would reduce vector mortal-ity risks associated with egg laying. Although evidence for *O. volvulus*-induced reduction of simuliid fecundity is scarce, we would predict that in unarmed blackflies, in which the proportion of ingested microfilariae that reach the thorax is larger and competition for resources possibly stronger, negative effects of infection on vector reproduction would be more pronounced than in vectors with a well-developed armature. In support of this conjecture are the results of Ham and Banya (1984), who infected *S. lineatum* Meigen (a species lacking cibarial projections) and *S. ornatum* s.l. (a species with toothed cibarium) with varying micro-filarial numbers of *O. lienalis* Stiles (an *Onchocerca* of cattle naturally transmitted by *S. ornatum*). In (unarmed) *S. lineatum*, and infecting the flies *per os*, fecundity was reduced by 21 to 76% of that of uninfected controls, with the magnitude of the effect depending on parasite concen-tration in the blood meal. A reduction by 21% was observed in (armed) *S. ornatum* but only when flies were fed on concentrations of microfilariae as large as 69,000 per ml. *S. lineatum* also showed a depression of oviposition rates when infected by *O. lienalis* larvae. Interestingly, the feeding rates in those groups of flies offered infected blood were reduced in several instances, suggesting the possibility of parasite avoidance. Intra-thoracic injection (which circumvents any earlier barrier to successful infection) also decreased fecundity, with reductions for *S. lineatum* depending on inocu-lum (36% for 10 microfilariae/fly; 54% for 50 microfilariae/fly). By contrast, reductions in *S. ornatum* s.l. were of a lower magnitude, reaching 13% when injected with 20 microfilariae/fly. Subsequently, Renshaw and Hurd (1994a,b) demonstrated that in the system *O. lienalis–S. ornatum*, and when 20 microfilariae were intra-thoracically injected immediately after a blood meal, there was a significant reduction of ovarian vitellin content; inocula lower than 20 did not exert a dose-dependent effect. Extending these exper-imental approaches to a range of natural *O. volvulus* vectors with well

characterised parasite success rates would be of interest for the elucidation of parasite-induced and density-dependent effects on components of vector fitness.

It is still unknown to what extent *O. volvulus* has adapted to selective pressures exerted by the various constraining and/or facilitating regulatory processes that operate in different vector species, or whether some of these processes have themselves evolved in response to parasite pressure. Besides, in many endemic areas there is more than one simuliid species that can act as a vector for the parasite, and although there may be a predominant species, species composition and abundance can change spatially and temporally (Grillet *et al.*, 2001; Vieira *et al.*, 2005; Vivas-Martínez *et al.*, 1998). Consequently, in a single endemic area selective pressures may operate in opposing directions. In simuliids with cibarial armatures and positive density dependence, increasing microfilarial availability or transmissibility to the flies may be an advantageous strategy for the parasite. Given that female worm fertility is already high (more than 1,000 microfilariae are produced daily per female; Schulz Key, 1990), and that their distribution in the skin is highly clumped (Kershaw *et al.*, 1954), the issue may be one of optimising allocation. One aspect that has received some attention is that of a positive correlation between the distribution of microfilariae and of vector bites along the human host's body, with for instance, microfilaridermia levels of the savannah strain of *O. volvulus* being higher in the lower half of the body (where *S. damnosum* preferentially bites; Renz and Wenk, 1983) and the converse being observed in Mesoamerica (Brandling-Bennett and Darsie, 1983; Kawabata *et al.*, 1980), where *S. ochraceum* frequently attacks the neck and upper torso (Dalmat, 1955).

If we accept the conjecture that the cibarial armature in simuliids is a trait evolved in response to haematophagia and not in response to parasite pressure (Reid, 1994), it may be advantageous for *O. volvulus* to exploit blackfly's salivary secretions, dermal microfilariae being attracted towards the inflicted wound. The paired blackfly comparisons listed in Section 11.1.2.2 seem to conform to expectation and rigorous testing of this hypothesis in a range of armed and unarmed species may be a fruitful research avenue. Even if armed simuliids ingest large numbers of microfilariae, the armature will protect the fly from excessive mortality, so the extrinsic incubation period can be completed and the parasite transmitted (Basáñez *et al.*, 1996). However, the selective advantage of parasite genes which may enhance microfilarial concentration to the site of the wound would be reduced by negative density dependence operating on microfilarial passage out of the stomach, limitation of thoracic development, or increased vector mortality in unarmed simuliids, which all decrease the probability an ingested microfilaria will develop into an L3 at high loads.

The interaction between the different positive (facilitating) and negative (constraining) density-dependent processes acting within the

parasite's life cycle may cause the maximum (overall) level of transmission to occur at intermediate skin microfilarial densities, with the distribution of those densities among and within human hosts being highly over-dispersed. Optimal density will also depend on the local biting rate (s) of the simuliid vector(s). Genetically structured mathematical models combining population dynamics and genetics may provide insights into evolutionary stable strategies for different *Onchocerca–Simulium* complexes. However, before we can implement such models much research is still required on the selective pressures that may operate for the evolution of susceptibility, resistance, virulence, infectivity and transmissibility in this system, the amount of variability present in the parasite's and vectors' genome for potentially relevant traits, and to demonstrate that parasites and simuliids have reciprocal effects on each other's phenotype and genotype (Webster *et al.*, 2004). Improving our understanding of the epidemiological and biomedical significance of co-evolution will require combining the phenomenological approaches of population and evolutionary biology with the search for mechanistic explanations underlying the parasitological and entomological patterns described in this paper, and making use of the extensive data that are becoming available on the molecular biology and genetics of parasite-vector interactions (Woolhouse *et al.*, 2002).

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