The advance of Muller's ratchet in a haploid asexual population: approximate solutions based on diffusion theory

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Summary

Asexual populations experiencing random genetic drift can accumulate an increasing number of deleterious mutations, a process called Muller's ratchet. We present here diffusion approximations for the rate at which Muller's ratchet advances in asexual haploid populations. The most important parameter of this process is $n_0 = N\,e^{-U/s}$, where N is population size, U the genomic mutation rate and s the selection coefficient. In a very large population, n_0 is the equilibrium size of the mutation-free class. We examined the case $n_0 > 1$ and developed one approximation for intermediate values of N and s and one for large values of N and s. For intermediate values, the expected time at which the ratchet advances increases linearly with n_0. For large values, the time increases in a more or less exponential fashion with n_0. In addition to n_0, s is also an important determinant of the speed of the ratchet. If N and s are intermediate and n_0 is fixed, we find that increasing s accelerates the ratchet. In contrast, for a given n_0, but large N and s, increasing s slows the ratchet. Except when s is small, results based on our approximations fit well those from computer simulations.

1. Introduction

The opposing forces of mutation and selection against deleterious mutations create at equilibrium a distribution of mutations across genomes in a population. If mutation rate is high and selection is weak, the number of mutation-free genomes can be small. Thus, random genetic drift in a finite population can lead to the loss of the zero (mutation-free) class. In the absence of back mutations and recombination, the loss is irreversible. As a result, asexual populations experiencing drift can accumulate an increasing number of deleterious mutations (Muller, 1964), a process often referred to as Muller's ratchet (Felsenstein, 1974). When the ratchet operates, sexual reproduction is advantageous because it makes up for the absence (or rarity) of back mutations by recreating mutation-free genomes through recombination between mutation-loaded genomes (Maynard Smith, 1978).

Although Muller's ratchet provides an appealing explanation for the evolution of sex, its action is not completely understood. No analytical solution for the advance of the ratchet is currently known. Haigh (1978) derived an analytical approximation for one phase of the ratchet, but resorted to Monte Carlo simulations to obtain a more complete solution. More recently, Charlesworth, Morgan and Charlesworth (1992), examined the ratchet and the effects of recombination on it in diploid populations, but they also relied on simulations.

Here we present the derivation of an approximate solution by the use of diffusion equations (Ewens, 1979; chapter 4). Our solution, which is for an asexual haploid population, gives a reasonably good fit to simulated results and provides a more mechanistic description of the advance of the ratchet than previous simulation approaches.

2. Haigh's model

We start with Haigh's (1978) model for the expected distribution of deleterious mutations across genomes in a haploid asexual population. It is assumed that organisms experience selection and then replicate. Mutations occur during replication and are Poisson distributed with a mean U (the genomic mutation rate). The deleterious effect of the mutations is

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assumed to be multiplicative across loci, i.e. the fitness of a genome with i mutations is $(1-s)^i$ and $0 < s < 1$. Thus, if $X_k(t)$ is the number of individuals with k mutations at generation t, the frequency of genomes with k mutations after selection and mutation is

$$p_k(t) = \sum_{j=0}^{k} X_{k-j}(t)(1-s)^{k-j} e^{-U} \frac{U^j}{j!} \bigg/ W, \qquad (1)$$

where

$$W = \sum_{i=0}^{\infty} X_i(t)(1-s)^i.$$

W/N is mean fitness and N is population size.

At equilibrium, the vector $\mathbf{X}(t) = (X_0(t), X_1(t), \ldots)$ equals the stationary distribution $\mathbf{n} = (n_0, n_1, \ldots)$, such that

$$n_k = \sum_{j=0}^{k} n_{k-j}(1-s)^{k-j} \frac{U^j}{j!}, \quad k = 0, 1, 2, \ldots.$$

The sole solution of this system of equations is (Kimura & Maruyama, 1966; Haigh, 1978)

$$n_k = N e^{-\theta} \theta^k / k!, \qquad (2)$$

where $\theta = U/s$. Because at equilibrium $X_0 = Np_0$, the equilibrium mean fitness is e^{-U} (see equation 1).

Haigh (1978) identified the equilibrium size of the zero-class $n_0 = Ne^{-\theta}$ as the most important single parameter of his model. As stated above, it is the loss of the zero-class in a finite population that starts Muller's ratchet. Once this class is lost, the one-class becomes the fittest class and its new (deterministic) equilibrium size is also n_0. However, the one-class may also be lost. If this occurs, the two-class becomes the fittest class and the process is repeated. Thus, there are in Haigh's model two phases to the advance of the ratchet. Haigh's Theorem 1 describes the essential part of this process as follows. In the first phase, immediately after the loss of the fittest class, the next fittest class is reduced from n_1 to a value close to n_0. In the second phase, after reaching a value close to n_0, the fittest class goes extinct. During the latter phase a complex rearrangement of the other classes takes place, such that at the end of the period the class means are close to n_i, $i \geq 1$.

Eventually, the number of generations required to complete Phases 1 and 2 approaches an equilibrium value, which we denote as T. The inverse of T is the rate of the ratchet. It is noteworthy that classes with few mutations (e.g. $X_0, X_1, X_2, \ldots$) may be extinct at this equilibrium, but the parameters n_0 and n_1 still govern the dynamics of Phases 1 and 2. Thus, in considering a single advance of the ratchet it is sometimes convenient to represent the recently lost fittest class, the new fittest class, the next fittest class, ... as $X_0, X_1, X_2, \ldots$, respectively. On other occasions, it is better to represent the new fittest class as X_0. The first approach is taken in Section (i) immediately below and the latter in Section (ii).

3. Diffusion approximations of Muller's ratchet

(i) $n_0 > 1$; N and s intermediate

Phase 1. Haigh's (1978) Theorem 1 describes the stochastic process $\mathbf{X}(t)$ after the loss of X_0 at time $t = 0$. At this time, the mean of the other classes, X_i where $i \geq 1$, is close to n_i. From Theorem 1, we have

$$E\{X_1(t) \mid \mathbf{X} + (0) = \mathbf{n}^+\} \approx n_1 \frac{\gamma(t)}{1 - e^{-\theta\gamma(t)}}, \qquad (3)$$

where $\gamma(t) = (1-s)^t$, $\mathbf{X}^+(0) = (X_1(0), X_2(0), \ldots)$ and $\mathbf{n}^+ = (n_1, n_2, \ldots)$. As $t \to \infty$ the trajectory approaches n_0, although very slowly at the end. The slowing down occurs, when γ reaches θ^{-1}, or equivalently, when X_1 reaches approximately $1.6n_0$. For the purpose of generating a diffusion approximation of the ratchet, it is natural to divide the diffusion domain of X_1 into the intervals $(1.6n_0, n_1)$ and $(0, 1.6n_0)$, which correspond to Phases 1 and 2, respectively.

To obtain the infinitesimal drift and diffusion operators (Ewens, 1979; chapter 4) for Phase 1, we compute the expected change in X_1 from generation t to $t+1$ by taking the time derivative of the right-hand side of equation (3). Because the right-hand side of equation (3) can be approximated by $n_1 \gamma(t)$ in Phase 1, we obtain

$$E\{X_1(t+1) - X_1(t) \mid X_1(t) = i\} \approx -si,$$
$$1.6n_0 < i < n_1. \quad (4a)$$

Furthermore, because of $i \ll N$,

$$\mathrm{Var}\{X_1(t+1) - X_1(t) \mid X_1(t) = i\} \approx i, \quad i < n_1. \qquad (4b)$$

Introducing $y = i/N$, X_1 can be rescaled to give, respectively, the drift and diffusion operators

$$a(y) \approx -sy, \quad 1.6e^{-\theta} < y < \theta e^{-\theta}, \qquad (5a)$$

$$b(y) \approx \frac{y}{N}, \quad y < \theta e^{-\theta}. \qquad (5b)$$

With these operators, the diffusion approximation (Ewens, 1979) estimates the expected time to complete Phase 1 as

$$T_1 \approx s^{-1} \ln \frac{\theta}{1.6}. \qquad (6)$$

Phase 2. The requirement that s and N are intermediate ensures that the two parameters are large, but not too large. The above Phase 1 analysis requires that s and N are sufficiently large such that the process X_1 is dominated by selection during Phase 1 and the Phase 2 interval $(0, 1.6n_0)$ is absorbing. The latter condition allows the two phases to be analyzed separately because once X_1 becomes less than $1.6n_0$ the process is trapped in Phase 2.

On the other hand, if s and N are not too large, Phase 2 is dominated by genetic drift. Hence, $a(y) = 0$, and $b(y)$ is given by equation (5b). Then, the

sojourn time in phase 2 is obtained in the diffusion approximation as

$$T_2 \approx 3 \cdot 2 N e^{-\theta}, \tag{7}$$

and the expected total time to loss of the fittest class is

$$T \approx s^{-1} \ln \frac{\theta}{1 \cdot 6} + 3 \cdot 2 N e^{-\theta}. \tag{8}$$

(ii) $n_0 > 1$; N and s large

If s is large, the time that the next fittest class spends in Phase 1 (after the loss of the fittest class) is likely to be short relative to that spent in Phase 2. If, in addition, N is large, the next fittest class also spends a long time close to the deterministic equilibrium n_0 and the latter is seen by the process as a reflecting barrier during Phase 2. Thus, T is determined primarily by the time required for the process to complete Phase 2, which is now more correctly the interval $(0, n_0)$, and the next fittest class is more conveniently defined as X_0.

From equation (1), the expected change of the X_0 process is

$$E\{X_0(t+1) - X_0(t) \mid X_0(t) = i\} = i \frac{e^{-U}}{\bar{w}} - i$$

$$= i \frac{\Delta \bar{w}}{\bar{w}}, \tag{9}$$

where $\Delta \bar{w} = e^{-U} - \bar{w}$, and $\bar{w}$ is the value of mean fitness in generation t.

We derived an approximation for $\Delta \bar{w}/\bar{w}$ by examining the response of the system to a perturbation by genetic drift. Assuming that the system is at equilibrium and that the perturbation is small, linear response theory (e.g. Risken, 1984; chapter 7) suggests the approximation

$$\Delta \bar{w} = C \left(1 - \frac{y}{y_0} \right), \tag{10}$$

where $y = i/N$ is the frequency of the zero-class, $y_0 = n_0/N = e^{-\theta}$ is its equilibrium frequency, and C is the generalized susceptibility of the system. Because N is assumed to be large, the perturbation by genetic drift is likely to be small.

C can be calculated from the properties of the system at equilibrium. Haigh (1978) has shown that if the system is close to equilibrium and N is large, mean fitness is relatively constant over time and close to its equilibrium expectation of e^{-U} (see above). This is because if the zero class ever drifts out of equilibrium such that its frequency becomes $y < y_0$, the reduction in mean fitness resulting from this departure from equilibrium is on the average compensated by an excess in mean fitness due to other classes. This excess results because the classes with the fewest mutations increase disproportionately.

However, whenever the ratchet advances with the loss of the zero class, such a compensation is no longer possible. Then, as the system approaches its new equilibrium, the excess in mean fitness created by the non-zero classes is also lost. Because mean fitness at the new equilibrium is $(1-s)e^{-U}$, the loss of mean fitness for each advance of the ratchet is $s e^{-U}$. Furthermore, because the contribution to mean fitness by the zero-class and the other classes before the advance of the ratchet is approximately equal (but of opposite signs), the reduction in mean fitness at the time of the loss of the zero class is approximately $\frac{1}{2}s e^{-U}$. Thus, for $y = 0$, it follows from equation (10) that

$$C = \Delta \bar{w} \approx \tfrac{1}{2} s e^{-U} \tag{11}$$

and

$$\frac{\Delta \bar{w}}{\bar{w}} \approx \tfrac{1}{2} s (1 - z)(1 - \tfrac{1}{2} s (1 - z))^{-1}, \tag{12}$$

where $z = y e^{\theta}$. Combining equations (9) and (12) gives the infinitesimal drift operator

$$a(z) \approx \tfrac{1}{2} s z (1 - z)(1 + \tfrac{1}{2}(1 - z)), \quad 0 < z < 1. \tag{13a}$$

Because the variance of $X_1(t)$ is the same as in case (i) (see equation 4b), the diffusion operator in the variable z is

$$b(z) \approx \frac{1}{N} e^{\theta} z, \quad 0 < z < 1. \tag{13b}$$

Thus, the diffusion approximation (Ewens 1979) is

$$T \approx 2N e^{-\theta} \int_0^1 z^{-1} e^{f(z)} \int_0^z e^{-f(y)} \, dy \, dz, \tag{14a}$$

where

$$f(z) = s N e^{-\theta} z \{(1 - \tfrac{1}{2} z) + \tfrac{1}{2} s (1 - z + \tfrac{1}{3} z^2)\}. \tag{14b}$$

4. Simulations of Muller's ratchet

A variety of assumptions were made in deriving our two analytical approximations of T. For example, in deciding whether to emphasize genetic drift over selection, we assumed N and s to be either large or intermediate. However, we are unable to define analytically how large these parameters have to be to obtain an adequate approximation. To test the accuracy of our approximations, we carried out computer simulations of Muller's ratchet and compared our analytical and simulated results.

(i) *Computer models*

Given N, U, s and a starting population (see below), we began a simulation by creating the offspring population in two steps. First, the expected frequency in the offspring population was generated deterministically by applying equation (1). Second, genetic drift was introduced by randomly drawing N offspring

from the new offspring frequencies, as described below. The resulting offspring population was then treated as the parent population for the next generation, and the process was repeated. All simulations

were carried for the number of generations required to observe one hundred advances of the ratchet.

Starting populations consisted of N individuals generated according to equation (2). Because such a population is at a deterministic equilibrium, we allowed the population to experience two advances of the ratchet before recording the simulation results. Simulation runs allowing more advances of the ratchet (before the population reached stochastic equilibrium and results were recorded) were investigated and found to give similar results. All simulations were started with a different random seed.

The programs were written in C and run on a MacIntosh SE/30. The random draw of the offspring population was accomplished by applying the multinomial pseudorandom number of generator of Devroye (1986). The program for the Devroye generator was written and kindly provided by J. Gillespie.

(ii) *Simulation results*

Our simulation results and the expected values based on our approximations (equations 8 and 14) are jointly presented in Figs. 1 and 2. We fixed values of U at 0·35 and 1·4 and s ranged from 0·025 to 0·2. N ranged from 30 to 10^8. Note that our results are
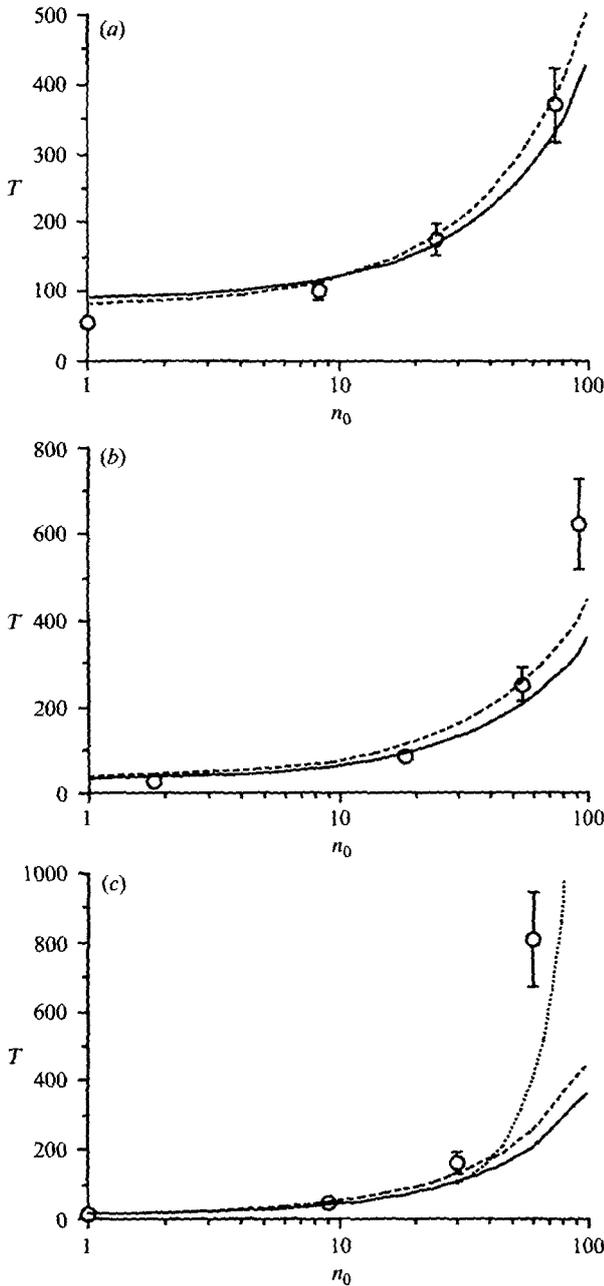
Fig. 1. Speed of Muller's ratchet as a function of n_0 for $U = 0.35$. T is the mean number of generations for one advance of Muller's ratchet. Parameters for the graphs are as follows: (a) $s = 0.025$, $\theta = 14$; (b) $s = 0.05$, $\theta = 7$; (c) $s = 0.1$, $\theta = 3.5$. Approximations of T are based on equation (8) (———); equations (14) (-----); equation (16) (---); simulated data (O). T based on equations (8) and (16) are drawn for values of $1 < n_0 < 100$. T based on equations (14) are only for sufficiently large values of selection coefficients and population sizes, i.e. n_0 larger than 20–30. Representative simulations are plotted as mean number of generations $\pm$ 2 s.e. No error bars are presented when they are smaller than the plotted symbol. The complete set of the simulation results is given in the Appendix.

Fig. 2. Speed of Muller's ratchet as a function of n_0 for $U = 1.4$. T is the mean number of generations for one advance of Muller's ratchet. Parameters for the graphs are as follows: (a) $s = 0.1$, $\theta = 14$; (b) $s = 0.2$, $\theta = 7$. See Fig. 1 for more details.

presented for T plotted against n_0 and that n_0 is varied by varying N.

All figures (except Fig. 1 a) show that our intermediate N and s approximation (equation 8) agrees well with the simulations for $1 < n_0 < 10$. When s is small, the agreement is good for values of n_0 as large as 30 (see Figs. 1 a and 1 b). However, there is a large discrepancy in the low n_0 range, when s becomes too small (Fig. 1 a). As expected, the approximation based on equations (14) does not agree well with simulations when N and/or s are too small (Figs. 1 c, 2 a and 2 b), even if n_0 is large (see Fig. 1 c). However, as both N and s increase, equations (14) fit the simulations better. In Fig. 2 b, theoretical results and simulations agree remarkably well.

5. Comparison with Haigh's estimate of T

For comparison, the parameter values used in our simulations were partly chosen to match those in Haigh's (1978) study. Haigh (1978) used separate approaches to obtain estimates of the rate of the ratchet. First, he derived analytically an estimate for the time required to complete Phase 1 as

$$T_1 \approx -\frac{\ln \theta}{\ln (1-s)}. \tag{15}$$

Equation (15) is similar to our diffusion approximation for intermediate values of s (equation 6), because of $\ln (1-s) \approx -s$. It is noteworthy that our diffusion approximation leads to a smaller estimate of T_1 than Haigh's result. This is because the diffusion approximation includes random genetic drift, whereas Haigh's description of Phase 1 is purely deterministic. However, for all of the parameter values that we examined (see Figs. 1 and 2), the values of T generated by combining equations (15) and (7) are indistinguishable from those by combining equations (6) and (7) (results not presented), because of $\theta \gg 1$.

Haigh did not attempt to derive an analytical solution for T_2 and, hence, T. Instead, he simulated the ratchet for $1 < n_0 < 14$ for a variety of U, s and N values, then fitted a multiple regression to the results and obtained the expression

$$T = 4N e^{-\theta} + 7 \ln \theta + 2s^{-1} - 20. \tag{16}$$

A comparison of values based on equation (16) (Figs. 1 and 2) shows that the latter equation matches very closely our equation (8). Both equations are good approximations of our simulated results when s and N are intermediate, but fail when the two parameters are large. The failure is not surprising considering that Haigh simulated the ratchet for only small n_0 values. Thus, for large values of s and N, equations (14) are the only expressions that give a reasonably good estimate of T.

6. Discussion

Previous studies have failed to produce an analytical solution for the speed of Muller's ratchet (Haigh, 1978; Pamilo, Nei & Li, 1987; Charlesworth *et al.* 1992). We present in this report two diffusion approximations for the advance rate of Muller's ratchet in a haploid asexual population. The approximations are for $n_0 > 1$ and two ranges of s and N values (intermediate and large). Except when s is small, estimates of T based on these analytical results fit well our simulation results of the ratchet. They also fit well the simulation results of Haigh (1978). Although other studies also conducted simulations, we were unable to compare their results with ours. Pamilo *et al.* (1987) examined the accumulation of mutations for individuals and not populations. Charlesworth *et al.*'s (1992) model was for diploid populations with and without recombination.

Although it is a desirable goal, we have not attempted in this study to obtain an analytical solution with recombination and diploidy. Instead, we have focused on Haigh's model, which divides the operation of the ratchet into two phases. If s is sufficiently strong, the two phases emerge because the boundary between them (n_0, or $1 \cdot 6 n_0$, depending on the approximation used) is absorbing. As a result, once Phase 2 is entered, the process cannot return to Phase 1. The reason why our approximations fail when s is small is because genetic drift can return the process to Phase 1 (and vice-versa) and the two phases are blurred. The latter is demonstrated in Fig. 1 a, where s is small; the theoretical and simulation results disagree in the linear n_0 range ($n_0 < 10$). In contrast, the fit is much better when s is larger in Fig. 1 c. If s is so small such that $n_0 < 1$, T is overestimated even more by equation (8) (results not presented). It would be desirable to find solutions for the ratchet for very small values of s.

The two-phase dynamics of the advance of Muller's ratchet shows that T is mainly characterized by the two parameters n_0 and s. As shown by our approximations, equation (8) and equations (14), n_0 is the most important parameter, as Haigh (1978) proposed, but T is additionally modulated by the value of s. The third parameter, $\theta = U/s$, is less important because it appears only logarithmically in equation (8) and is not present in equations (14).

The parameter n_0 governs the stochastic loss of the fittest class through genetic drift. On the other hand, s tends to stabilize the system at the stationary distribution given by equation (2). As a result, the importance of drift and selection varies depending on the phase and on whether N and s are intermediate or large. When N and s are intermediate, the dynamics of Phase 1 is essentially deterministic and driven by selection, while that of Phase 2 is dominated by drift. On the other hand, when s and N are large, selection dominates both phases. However, the strength of

selection drives the system quickly through Phase 1 and most of the time is spent in Phase 2. Drift still operates during Phase 2, but its role is primarly one of perturbing the system away from equilibrium and triggering the loss of the fittest class. Selection is counteracting drift by driving the fittest class back to the deterministic equilibrium value n_0.

It is noteworthy that, for a given value of n_0, increasing s can both speed up or slow down the ratchet. This results from the varying importance of genetic drift and selection in Phases 1 and 2. If N and s are intermediate and n_0 is fixed, stronger selection accelerates the ratchet because Phase 1 is completed sooner (equation 6). In contrast, with large N and s and a given value of n_0, the ratchet is slowed because Phase 2 is prolonged by the increased selection (see equation 13a). As equations (14) suggest, the slowing down occurs more or less exponentially with increasing values of s. This dramatic effect is illustrated by the much greater value of T for n_0 between 50 and 70 when s is increased between Figs. 2a and 2b.

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Appendix

Complete data set of estimates for T as a function of N, s and U. Values from simulations are mean $\pm$ S.E.

$N \times 10^{-3}$	s	U	θ	n_0	Estimates of T based on Equation (16)	Equation (8)	Equations (14)	Simulations
1 000	0·025	0·35	14	0·8	81·8	89·4	—	49·8 ± 2·5
1 203				1·0	82·5	90·0	—	54·7 ± 3·2
3 000				2·5	88·5	94·7	—	68·6 ± 4·3
5 000				4·2	95·1	100·1	—	81·6 ± 5·3
10 000				8·3	111·7	113·4	—	101·4 ± 6·2
20 000				16·6	145·0	140·0	—	161·0 ± 13·4
30 000				25·0	178·3	166·6	—	175·3 ± 11·1
40 000				33·3	211·5	193·2	—	202·6 ± 14·9
50 000				41·6	244·8	219·8	—	266·3 ± 21·1
60 000				49·9	278·0	246·4	—	282·0 ± 20·7
70 000				58·2	311·3	273·0	—	303·9 ± 24·0
90 000				74·8	377·8	326·2	—	368·5 ± 26·2
1	0·05	0·35	7	0·9	37·3	32·4	—	19·5 ± 1·4
2				1·8	40·9	35·4	—	28·2 ± 1·9
5				4·6	51·9	44·1	—	40·4 ± 2·4
10				9·1	70·1	58·7	—	63·2 ± 4·9
15				13·7	88·3	73·3	—	68·9 ± 5·2
20				18·2	106·6	87·9	—	85·0 ± 6·6
30				27·4	143·1	117·1	—	128·8 ± 10·7
40				36·5	179·5	146·2	—	177·9 ± 11·4
50				45·6	216·0	175·4	—	214·9 ± 17·2
60				54·7	252·5	204·6	—	251·9 ± 20·6
70				63·8	289·0	233·8	—	314·5 ± 22·6
80				73·0	325·4	263·0	—	427·0 ± 32·8
90				82·1	361·9	292·1	—	470·8 ± 41·0
100				91·2	398·4	321·3	—	624·9 ± 51·7
0·033	0·1	0·35	3·5	1·0	12·8	11·0	—	10·5 ± 1·0
0·1				1·5	14·8	12·7	—	12·0 ± 1·1

Appendix (*cont.*)

$N \times 10^{-3}$	s	U	θ	n_0	Estimates of T based on			
					Equation (16)	Equation (8)	Equations (14)	Simulations
0·15				3·0	20·9	17·5	—	21·1 ± 2·1
0·2				6·0	32·9	27·2	—	33·8 ± 2·7
0·3				9·1	45·0	36·8	—	43·2 ± 3·7
0·4				12·1	57·1	46·5	—	66·8 ± 5·0
0·5				15·1	69·2	56·1	—	69·6 ± 5·8
0·6				18·1	81·2	65·8	—	86·9 ± 7·5
0·7				21·1	93·3	75·5	—	107·7 ± 8·6
0·8				24·2	105·4	85·1	—	133·0 ± 10·4
0·9				27·2	117·5	94·8	—	143·5 ± 12·7
1·0				30·2	129·6	104·5	100·6	162·2 ± 17·3
1·1				33·2	141·6	114·1	117·5	207·8 ± 15·8
1·2				36·2	153·7	123·8	136·4	253·6 ± 21·8
1·3				39·3	165·8	133·5	157·6	241·4 ± 21·1
1·4				42·3	177·9	143·1	181·2	309·4 ± 26·6
1·5				45·3	190·0	152·8	207·8	364·7 ± 33·3
2·0				60·4	250·4	201·1	400·0	809·3 ± 68·5
1 000	0·1	1·4	14	0·8	21·8	24·4	—	20·2 ± 1·3
2 000				1·7	25·1	27·0	—	25·8 ± 1·6
5 000				4·2	35·1	35·0	—	32·9 ± 2·1
10 000				8·3	51·7	48·3	—	46·8 ± 3·2
20 000				16·6	85·0	74·9	—	77·9 ± 5·6
30 000				25·0	118·3	101·5	75·1	111·0 ± 8·9
40 000				33·3	151·5	128·1	117·8	160·5 ± 15·4
50 000				41·6	184·8	154·7	175·5	233·5 ± 17·9
60 000				49·9	218·0	181·3	254·7	281·7 ± 24·8
70 000				58·2	251·3	208·0	364·5	340·7 ± 30·6
80 000				66·5	284·6	234·6	518·5	455·6 ± 35·2
90 000				74·8	317·8	261·2	736·3	596·5 ± 62·9
1	0·2	1·4	7	0·9	7·3	10·3	—	8·7 ± 0·6
2				1·8	10·9	13·2	—	13·9 ± 1·1
5				4·6	21·9	22·0	—	20·1 ± 1·6
10				9·1	40·1	36·6	—	39·7 ± 2·6
15				13·7	58·3	51·2	—	65·7 ± 5·5
20				18·2	76·6	65·7	—	79·7 ± 6·3
25				22·8	94·8	80·3	—	140·7 ± 13·2
30				27·4	113·1	94·9	163·9	217·1 ± 21·0
33				29·6	122·2	102·2	200·2	266·5 ± 23·1
35				31·9	131·3	109·5	244·3	300·5 ± 25·6
40				36·5	149·5	124·1	363·5	433·7 ± 38·5
50				45·6	186·0	153·3	810·3	917·4 ± 78·3
60				54·7	222·5	182·5	1843·6	1910·0 ± 196·3