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## Epidemiology of Poly-Parasitism*

### II. Types of Combinations, Relative Frequency and Associations of Multiple Infections

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Summary

Frequency distributions, including all of the observed types of combinations of multiple infections with different helminths and protozoa are presented for sample villages of the African Savannah and of the trans-Andean part of Peru. Measurements of correlation between these infections based on properties of the multivariate, multinomial distribution are calculated to show age and sex patterns of association in different population samples. A correlation matrix for combined infections with *Dipetalonema perstans, D. streptocerca* and *Loa loa,* in villages in the rain forest of Zaire indicates that there is a statistically significant association between the two species of *Dipetalonema* and *L. loa.* There is also a strong association between the numbers of the microfilariae of *D. perstans* and *D. streptocerca* in multiply infected individuals. This correlation is strong only in the rain forest; it is insignificant in the other ecological zones of Bas-Zaire included in the study. The data suggest that there may be selective host factors that influence the extent, distribution and the types of multiple infections in a community.

**Zur Epidemiologie der Polyparasitosen**
**2. Kombinationstypen, relative Häufigkeiten und statistische Zusammenhänge bei Mischinfektionen**

Für alle in Stichproben-Dörfern der afrikanischen Savanne und des Trans-Andenteiles von Peru beobachteten Kombinationstypen von Polyparasitosen mit unterschiedlichen Helminthen und Protozoen werden Häufigkeitsverteilungen dargestellt. Um die Zusammenhangmuster für Alter und Geschlecht zu zeigen, sind auf der Basis einer mehrdimensionalen polynomischen Verteilung korrelationsstatistische Messungen durchgeführt worden. Eine Korrelationsmatrix für kombinierte Infektionen mit *Dipetalonema perstans, D. streptocerca* und *Loa loa* im Regenwald von Zaire beweist, daß ein statistisch signifikanter Zusammenhang zwischen den beiden Spezies *Dipetalonema* und *Loa loa* existiert. Zwischen den Mikrofilarienmengen von *D. perstans* und *D. streptocerca* besteht bei multipel infizierten Individuen ebenfalls ein gesicherter Zusammenhang – jedoch nur im Regenwald, nicht in anderen ökologischen Zonen dieser Studie in Zaire. Die Daten lassen darauf schließen, daß selektive Wirtsfaktoren existieren, die das Ausmaß, die Verteilung und die Typen multipler Infektionen in einer Gemeinde beeinflussen.

## Introduction

In a previous paper we presented data on poly-parasitism for different endemic diseases as observed in 13 villages of ecologically contrasting areas in three countries (Buck et al. 1978). In these villages, it was found that multiple infections with two or more parasites and with other infectious agents were the rule rather than the exception. The observed combinations of parasitic infections in the African villages included some of the most important tropical diseases such as malaria, schistosomiasis and filarial infections. The previous paper gave descriptions of the age and sex patterns of multiple parasitic infections as observed in the residents of selected communities of Chad, Peru and Afghanistan.

The present paper shows the relative frequencies of poly-parasitism according to the types of combination found in two villages of Chad and one of Peru. The closeness of association

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between co-endemic diseases is calculated for individual age groups and by sex. Included in the analyses are also population samples from three ecologically different parts of the lower Congo in Zaire where multiple infections with as many as five different species of filarial worms are found. For these villages, correlation coefficients for combined infections with different types of microfilariae are determined. Also calculated are deviations of the observed from the expected random distribution of combined infections with filarial parasites in population samples. The data from Zaire (Fain 1974, Fain et al. 1974) also permit an analysis of association to be made between the *numbers* of microfilariae of different species found together in the same persons.

## Material and Methods

The descriptions of the population samples and of the diagnostic methods employed in the three-country study and in the investigations of filariasis in Zaire, are given in a previous paper (Buck et al. 1978). The analysis of association among infectious states is based on properties of the multivariate, multinomial distribution described by Solomon (1960). In our calculations, a correlation matrix is constructed from the available prevalence data of the parasitic infections. An example is shown in Table 4 of this paper. For more detailed studies of the age patterns of association between diseases, the correlation co-efficients of individual age groups are presented. For testing the statistical significance of the observed sex differences, Spearman's rank correlation coefficients (1971) are calculated.

It should be kept in mind that the correlation coefficients are based on prevalence data that were obtained at a given point in time, irrespective of seasonal variations and of other dynamic factors that might influence the patency of an infection. For this reason, the correlation co-efficients listed in this paper measure only the extent to which the various types of infections are found together; they say nothing about whether they develop together.

For the comparison of expected and observed values shown in Table 5, we assume that the three co-en-

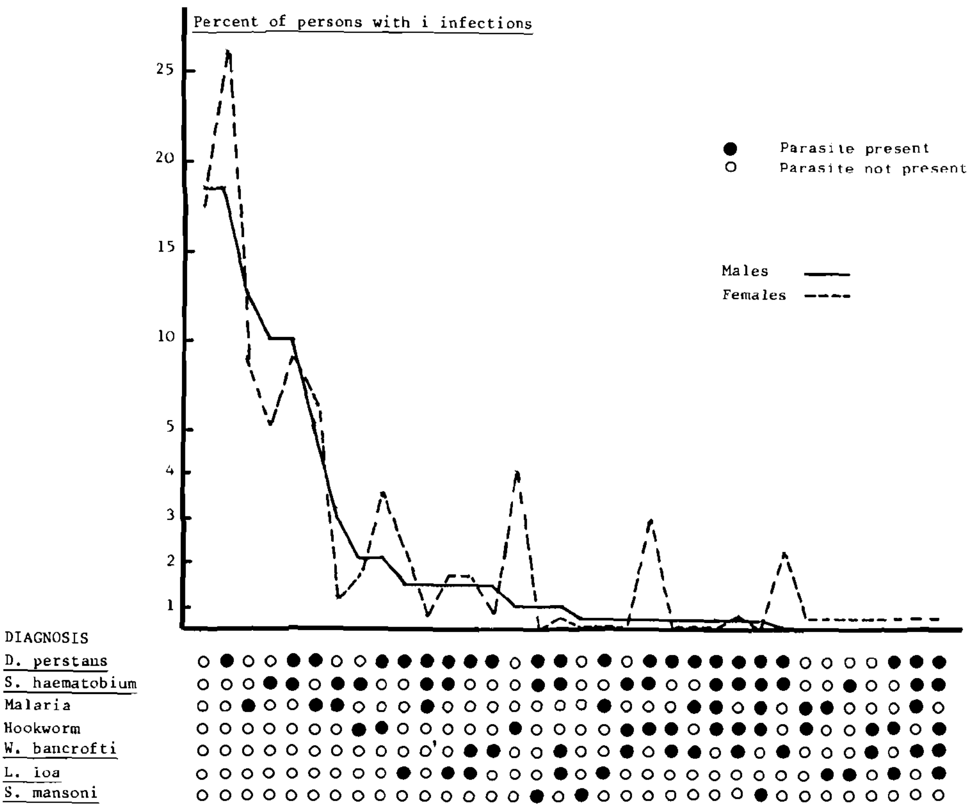


Fig. 1. Frequency distribution of single and multiple infections with malaria, *D. perstans*, *W. bancrofti*, *L. loa*, *S. haematobium*, *S. mansoni* and hookworm in the village of Ouarai (n = 356), Chad, by sex (m = 173, multi f = 183)

demic filarial infections are independent and that they have a multinomial distribution. The statistical significance of the difference between the observed and expected distributions of single and multiple infections is determined in a chi-square test.

## Results

Figure 1 shows the frequency distributions of the various types of combination of multiple infections with seven different parasites as observed in a village of the African Savannah. The seven infections include three filarial infections, i.e. *Dipetalonema perstans, Wuchereria bancrofti* and *Loa loa,* two types of schistosomiasis, namely, *Schistosoma mansoni* and *S. haematobium,* malaria and hookworm. The relative prevalence of the individual infections is shown in Table 4, of a previous paper (Buck et al. 1978). Based on the observations of actual cases only 34 of the 128 possible combinations for multiple infections with seven agents are shown here.

The figure indicates that less than 20% of the residents have neither one of the seven infections as determined by routine examinations of single blood, stool and urine specimens on examination day. *Dipetalonema perstans* is the most frequently found individual infection in the village; most of the combined infections involve agents of recognized pathogenicity. The general patterns of poly-parasitism in the village are similar for the two sexes.

Another example of poly-parasitism in an African village is shown in Figure 2 including malaria, four different filarial parasites (*Onchocerca volvulus, D. perstans, W. bancrofti* and *L. loa),* two types of schistosomiasis (*S. haematobium, S. mansoni*) and hookworm. Most frequent among the 37 observed disease combinations are double infections with *O. volvulus* and *S. mansoni,* only 9% of the females and 15% of the males are found uninfected. Advanced onchocerciasis is clinically the most

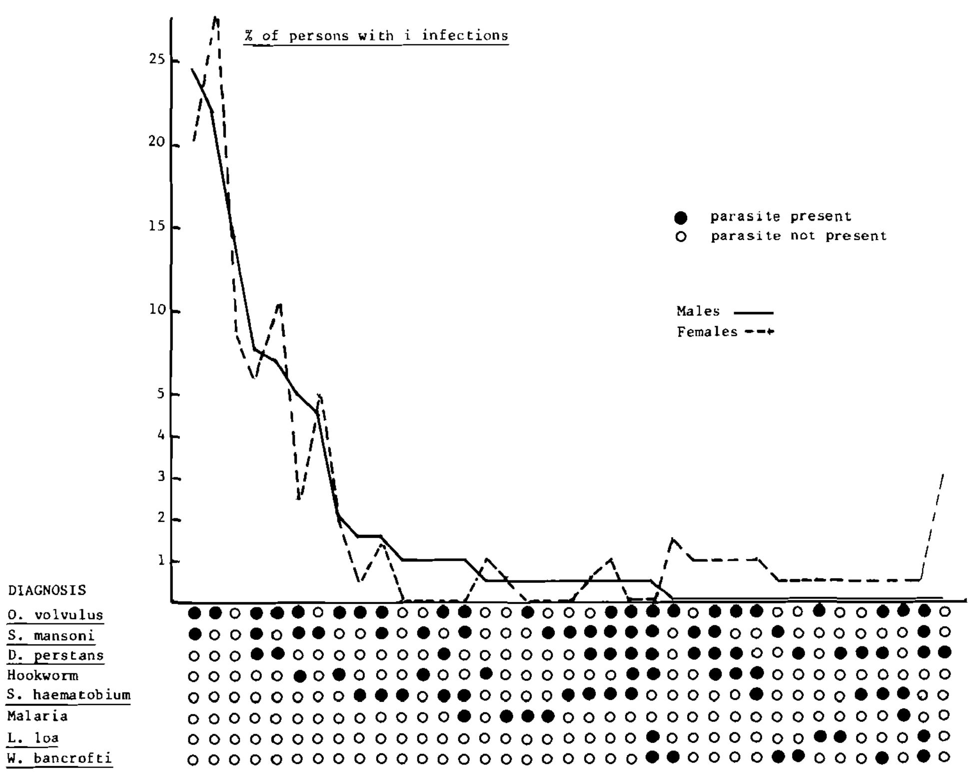


Fig. 2. Frequency distribution of single and multiple infections with malaria, *O. volvulus, D. perstans, L. loa, W. bancrofti, S. mansoni, S. haematobium* and hookworm in the village of Ouli Bangala (n = 379), Chad, by sex (m = 169, f = 210)

conspicuous of the seven locally important parasitic infections in the village. Depicted in Figure 2 are only those disease combinations for which cases are actually seen. Theoretically, there are 256 different possibilities of how eight infections can combine. With one exception, all of the infections with *L. loa* and *W. bancrofti* in this village are found in women. This observation could be related to certain peculiar risks of exposure to infection of the women or could indicate that the infection had been acquired at another place of residence before marriage to their present husbands.

An example of poly-parasitism is also shown in Figure 3 for five species of intestinal parasites that are of public health importance to the residents of a village in the trans-Andean jungle of Peru. Clinically, the most severe and prevalent of the parasitic diseases is severe hookworm anaemia aggravated by malnutrition and by the habitual use of chewing large quantities of coca leaves (Buck et al. 1968, 1970). The relatively small number of five co-endemic infections in this example permits that all of the possible combinations of multiple infections with the five agents be presented. As can be seen, the two distribution curves for males and females are almost congruent. The most frequently observed combinations are triple infections with *Ascaris lumbricoides, Trichuris trichiura* and hookworm. The percent of persons without any of those five infections is negligible in this village where even infants and many of the toddlers are already haveily parasitized.

As a measurement of association between individual pairs of parasitic infections, correlation co-efficients are calculated according to the method described above. For sex comparison these values are ranked from the highest positive to the lowest negative in the males

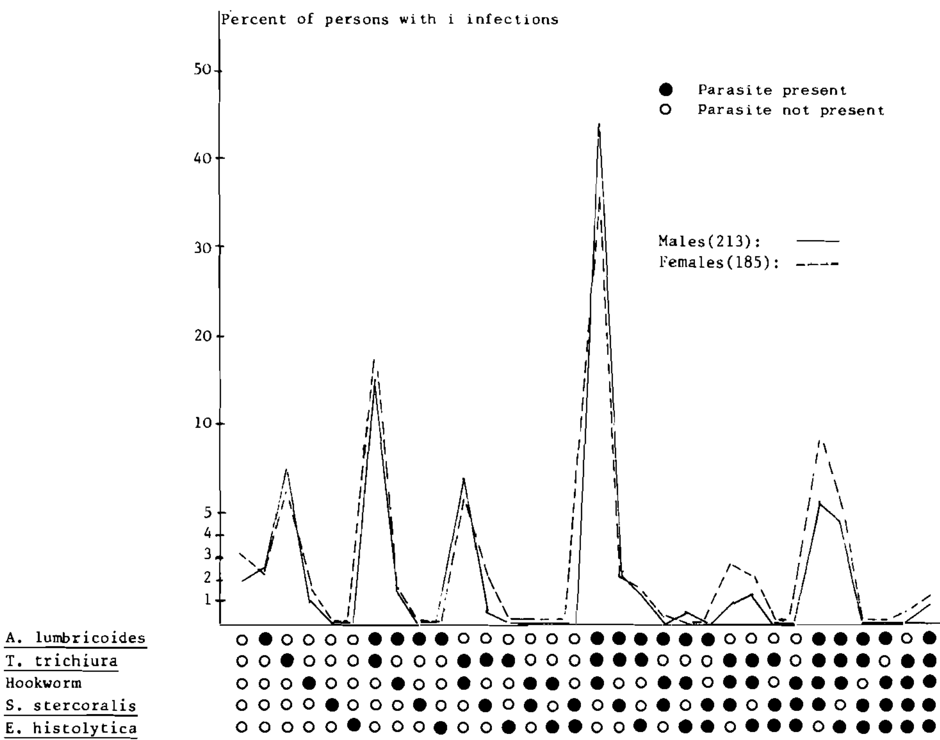


Fig. 3 Frequency distribution of single and multiple infections with *A. lumbricoides, T. trichiura, hookworm, S. stercoralis* and *E. histolytica* in the village of Cachicoto (Monzon), Peru, by Sex (M = 213, F = 184)

and then compared with those of the corresponding disease combination in the females. Table 1 shows that the relatively highest correlation co-efficient in both sexes is found for multiple infections with filarial worms and the lowest for pairs of combined infections in which malaria is one of the partners. The general ranking pattern of the correlation co-efficients for the paired infections is similar for males and females. Statistically this is indicated by the rank correlation co-efficient of 0.8268, which is significant at the 5% level.

There is the possibility that a negative association between two infections, if calculated for the total population sample. could have resulted from differences in the age patterns, one infection having its highest prevalence in early childhood as for example, hyperendemic malaria, and the second characterized by a steady increase in prevalence with progressing age, as found in endemic infections with *D. perstans* and *L. loa*. The influence of age on the correlation co-efficients is examined in Table 2 (p. 142). It is interesting to note that in the dry season when the studies were conducted the correlation between parasitaemia of malaria and *L. loa* microfilaraemia remains negative throughout the entire age range of the population. In contrast, the association between parasitaemia of malaria in the dry season and of microfilaraemia with *D. perstans* is positive in all but one of the 10-year age groups. It is also interesting to note that the positive associations between *L. loa* and *D. perstans*, and between *L. loa* and *W. bancrofti*, remain strong throughout life.

It should be emphasized that these correlation co-efficients are relatively crude indicators of association between the chronic parasitic infections with their often pronounced seasonal variations. The data were acquired in a cross-sectional survey. It would be interesting to study variations of these co-efficients longitudinally and determine the influence of the seasons, the incidence of intercurrent disease and the dynamics of morbidity and mortality patterns for pairs of these parasitic infections.

Table 3 (p. 142) demonstrates the correlation co-efficients for the ten pairs of combined infections with five different intestinal parasits. The co-efficients of the males are arranged by rank from positive to negative and are compared with those of corresponding disease combinations of the females. All associations are relatively weak and reach statistical significance only in a few combinations. Comparison of the correlation co-efficients for sex differences reveals general similarities between the two groups.

Table 1 Association between six parasitic infections, by sex: correlation coefficients for infections with two parasites, Ourai, Chad (N = 356)

| Correlation coefficient for pairs of infections | Males (N = 173) | | Femals (N = 183) | |
|---|---|---|---|---|
| | r | Rank | r | Rank |
| *L. loa* – *W. bancrofti* | +.3537* | 1 | +.3084* | 1 |
| *L. loa* – *D. perstans* | +.2653* | 2 | +.1430 | 3 |
| *D. perstans* – *W. bancrofti* | +.2335 | 3 | +.1331 | 4 |
| *S. haematobium* – hookworm | +.1264 | 4 | +.1014 | 5 |
| Hookworm – *W. bancrofti* | +.1264 | 5 | +.0819 | 7 |
| *D. perstans* – hookworm | +.0875 | 6 | –.0383 | 10 |
| *L. loa* – *S. haematobium* | +.0399 | 7 | +.0709 | 8 |
| *S. haematobium* – *W. bancrofti* | +.0228 | 8 | +.1847* | 2 |
| *D. perstans* – *S. haematobium* | +.0032 | 9 | +.0936 | 6 |
| Malaria – hookworm | –.0474 | 10 | –.1539 | 14 |
| Malaria – *W. bancrofti* | –.0553 | 11 | –.0134 | 9 |
| *L. loa* – hookworm | –.0902 | 12 | –.0702 | 12 |
| Malaria – *S. haematobium* | –.0919 | 13 | –.1354 | 13 |
| Malaria – *L. loa* | –.0985 | 14 | –.0485 | 11 |
| Malaria – *D. perstans* | –.1112 | 15 | –.1677* | 15 |

*Significant at 5% level
Spearman's rank correlation coefficient $r_S = .8268$ (z = 3.1)

Table 2 Association between six parasitic infections [1], by age: correlation coefficients for infections with two parasites, Ourai, Chad (N = 356)

| Infection combination | Age in years 0–9 | 10–19 | 20–39 | 40+ | Total |
|---|---|---|---|---|---|
| Malaria – *L. loa* | –.3261* | –.1393 | –.0929 | –.1265 | –.0745 |
| Malaria – *D. perstans* | –.1102 | +.1694 | +.0490 | +.1092 | –.1425* |
| Malaria – *S. haematobium* | –.0724 | –.2728* | –.0754 | –.1265 | –.1072* |
| Malaria – hookworm | –.1144 | –.0589 | –.1294 | +.0325 | –.1072* |
| Malaria – *W. bancrofti* | +.0941 | +.1968 | +.0626 | –.1265 | –.0345 |
| *L. loa – D. perstans* | +.2217* | +.2542 | +.0449 | +.1726 | +.2006* |
| *L. loa – S. haematobium* | +.0708 | +.0329 | +.0360 | +.0663 | +.0529 |
| *L. loa* – hookworm | +.0673 | –.1318 | –.0929 | –.1030 | –.0445 |
| *L. loa – W. bancrofti* | +.4540* | +.1826 | +.1328 | +.4666* | +.3282* |
| *D. perstans – S. haematobium* | +.1879* | –.1827 | –.1519 | +1726 | +.0420 |
| *D. perstans* – hookworm | +.0184 | + 1451 | –.1812 | –.2159 | +.0227 |
| *D. perstans – W. bancrofti* | +.1472 | +.1825 | +.1316 | + 0240 | +.1821 |
| *S. haematobium* – hookworm | +.1885* | +.1987 | +.0019 | +.0203 | +.1067 |
| *S. haematobium – W. bancrofti* | +.3013* | +.1561 | –.0376 | –.0669 | +.1017 |
| Hookworm – *W. bancrofti* | +.1394 | +.2417* | +.0626 | +.0203 | +.1011 |

*Significant at 5% level
[1]Malaria: 21.1% (parasitaemia); *L.loa:* 7.9%; *D. perstans:* 55.1%; *W. bancrofti:* 7.0%; *S. haematobium:* 30.9%; Hookworm: 12.1%.

The correlation matrix for combined infections with *D. perstans, D. streptocerca* and *L. loa* in villages of the rain forest region of Zaire is given in Table 4 (p. 143). Also shown in the Table are the first-order partial correlations $r_{xy.z}$ and the multiple correlation $R_{1.23}$. The figures indicate that there is a statistically significant association between infections with the two species of *Dipetalonema*, and that *L. loa* occurs more frequently in the presence of double infections with *D. perstans* and *D. streptocerca* than in combination with only one of these two infections. This is also evident from Table 5 (p. 143) where the observed and expected distribution of polyparasitism with the three filarial worms is shown for each sex. There are observed surpluses at both extremes, namely of triple infections and of persons without any of the three parasites. These excesses are compensated by relatively smaller numbers of persons who have double infections with *D. perstans* and *L. loa* and single infections with either of the two species of *Dipetalonema.* A possible explanation for the observed tendency to double infections with *D. perstans* and *D.*

Table 3 Association between 5 intestinal parasitic infections, by sex: correlation coefficients for infections with two parasites, Cachicoto, Peru (N = 397)

| Correlation coefficients for pairs of infections | Males (N = 213) r | Rank | Females (N = 184) r | Rank |
|---|---|---|---|---|
| Ascaris – hookworm | +.1672* | 1 | +.1797* | 4 |
| Trichuris – hookworm | +.1290 | 2 | +.1926* | 3 |
| Hookworm – *E. histolytica* | +.1129 | 3 | +.2174* | 2 |
| Ascaris – Trichuris | +.1068 | 4 | +.2233* | 1 |
| Trichuris – Strongyloides | +.1000 | 5 | +.1443 | 5 |
| Trichuis – *E. histolytica* | +.0939 | 6 | +.0953 | 6 |
| Hookworm – Strongyloides | +.0699 | 7 | +.0888 | 7 |
| Ascaris – Strongyloides | +.0588 | 8 | –.0570 | 10 |
| Ascaris – *E. histolytica* | +.0051 | 9 | –.0447 | 9 |
| Strongyloides – *E. histolytica* | –.0034 | 10 | +.0564 | 8 |

*significant at 5% level
Spearman's rank correlation coefficient $r_s$ = 0.83; (z = 2.49)

Table 4 Correlation matrix of combined infections with three filarial parasites in villages of Zaire (N = 248)

| | *D. perstans* | *D. streptocerca* | *L. loa* |
|---|---|---|---|
| *D. perstans* | 1.0 | .39720* | .03911 |
| *D. streptocerca* | | 1.0 | .24711 |
| *L. loa* | | | 1.0 |

*Significant at 5% level

$r_{12.3}$ = .40034; $r_{23.1}$ = .25253; $r_{13.2}$ = .00639; $R_{1.23}$ = .40185

Table 5 Observed and expected numbers of infections in 3 villages of the rain forest of Bas-Zaire where *Dipetalonema perstans, Dipetalonema streptocerca* and *Loa loa* are co-endemic

| Males | $p_1 p_2 p_3$ | $p_1 p_2 q_3$ | $p_1 p_3 q_2$ | $p_2 p_3 q_1$ | $p_1 q_2 q_3$ | $p_2 q_1 q_3$ | $p_3 q_1 q_2$ | $q_1 q_2 q_3$ | N | $Chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Observed | 31 | 51 | 1 | 3 | 14 | 6 | 1 | 7 | 114 | 17.43 |
| Expected | 24.4 | 53.0 | 6.2 | 4.3 | 13.4 | 9.3 | 1.1 | 2.3 | 114.0 | |
| Females | | | | | | | | | | d.f.4, P = < 0.01 |
| Observed | 23 | 52 | 7 | 4 | 23 | 1 | 4 | 20 | 134 | 33.3 |
| Expected | 17.8 | 44.9 | 12.0 | 4.9 | 30.3 | 12.4 | 3.3 | 8.4 | 134.0 | |
| | | | | | | | | | | d.f.4, P = < 0.01 |

$p_1$ = *D. perstans;* $p_2$ = *D. streptocerca;* $p_3$ = *L. loa*

*streptocerca* might be found in their being transmitted by the same local vector, *Culicoides grahami.*

The observation that in certain parts of Zaire double infections with *D. perstans* and *D. streptocerca* occur more frequently than expected by chance alone has led us to further analyse the data collected by *Fain* et al. (1974) and to study associations between the numbers of microfilariae of these two species of *Dipetalonema* in specimens of dermal fluid. Standardization of the volume of dermal fluid obtained by skin scarification is not possible. This methodological deficiency could have introduced a certain degree of bias. However, one would expect this bias to be similar in each of the three types of habitats listed in Fig. 4 (p. 144). The results show that there is a strong association between the numbers of microfilariae of *D. perstans* and of *D. streptocerca* in the rain forest. By comparison, the corresponding correlation co-efficents in the other two types of habitat are weak.

## Discussion

This paper has presented data on the quantitative relationship between different combinations of parasitic infections. In many villages of Africa and also in other parts of the tropics, there is a great variety of multiple infections with parasites that cause tropical diseases of major public health importance. There is still little understanding of their interaction and of their medical and economical importance, including their role in affecting disease control programmes. The available findings suggests that the distribution of persons with multiple infections is not strictly random and that there may be selective host factors that determine the extent and distribution of poly-parasitism in a community.

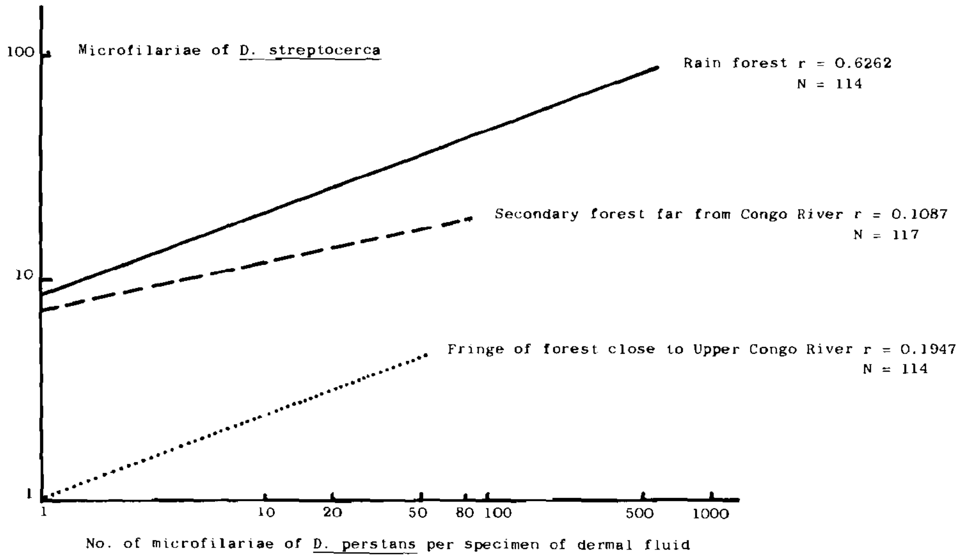


Fig. 4 Association between the numbers of microfilariae of two different species in the dermic fluid[1] of persons with double infections of *Dipetalonema perstans* and *Dipetalonema streptocerca* in three ecologically different parts of Bas-Zaire

[1] Obtained by skin scarification

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