SHORT COMMUNICATION

# Distinction of Males of the *Lutzomyia intermedia* (Lutz & Neiva, 1912) Species Complex by Ratios between Dimensions and by an Artificial Neural Network (Diptera: Psychodidae, Phlebotominae)

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*The females of the two species of the* Lutzomyia intermedia *complex can be easily distinguished, but the males of each species are quite similar. The ratios between the extra-genital and the genital structures of* L. neivai *are larger than those of* L. intermedia s. s., *according to ANOVA. An artificial neural network was trained with a set of 300 examples, randomly taken from a sample of 358 individuals. The input vectors consisted of several ratios between some structures of each insect. The model was tested on the remaining 58 insects, 56 of which (96.6%) were correctly identified. This ratio of success can be considered remarkable if one takes into account the difficulty of attaining comparable results using traditional statistical techniques.*

Key words: Psychodidae - Phlebotominae - artificial neural networks

Some Phlebotomine sand fly species can only be distinguished by one sex. Although males are clearly distinct, females of closely related species may be very similar, as with the *Lutzomyia wellcomei*/*L. complexa* complex (Frahia et al. 1971). Since these species are biologically and epidemiologically different, their correct identification is very important. Multivariate analysis was utilized to classify females of these species (Lane & Ready 1985) as well as those of some species in Venezuela (Añez et al. 1997).

Females of the *L. intermedia* species complex [*L. intermedia s. s.* and *L. neivai* (Pinto, 1926)] have been incriminated as vectors of parasites causing dermal leishmaniasis in South America (see Marcondes et al. 1998a, b). Both species seem to be parapatric throughout most of their range, but they were collected in a municipality in the Ribeira River Valley, in the southeastern region of the State of São Paulo, Brazil (Marcondes et al. 1998a).

The distinction between females of *L. intermedia* and *L. neivai* by morphological criteria (spermathecae, spermathecae ducts and cibarial teeth) is obvious (Marcondes 1996), however, males of both species are very similar. Some differences between males of both species, grouped by the origin and the associated females, were listed (Marcondes et al. 1998b). Due to the above cited parapatric distribution this could be useful.

The ratios between nine extra-genital (for the meaning of the Greek letters, see Forattini 1973) and six genital structures of 358 males from Brazil, Paraguay, Argentina and Bolivia were calculated. Insects from Pariqüera Açu in the Ribeira River Valley, State of São Paulo, where both species have been found, were not included, because it would have been impossible to assign them to one species or the other. The male insects were identified as either *L. intermedia* (208 insects) or *L. neivai* (150 insects) based on the morphology of the associated females.

All the above ratios between extra-genital and genital structures were significantly higher at a significance of 0.1% when *L. neivai* was compared to *L. intermedia* (Table). However, the overlap between the measurements was too high to use any of them for a unequivocal distinction. Each collection of these ratios, along with the corresponding probable classification of the

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TABLE

Ratios between extra-genital and genital dimensions of *Lutzomyia intermedia* and *L. neivai*

| | *Lutzomyia intermedia*/*L. neivai* | | |
|---|---|---|---|
| Structures | Samples | Means | *F* |
| Palp3/coxite | 184/117 | 0.466/0.526 | 71.9 |
| Palp3/style | 172/117 | 0.944/1.024 | 88.2 |
| Palp3/paramere | 160/112 | 0.725/0.764 | 24.4 |
| Palp3/lateral lobe | 172/116 | 0.472/0.521 | 124.5 |
| Palp3/pump | 136/117 | 0.636/0.726 | 162.8 |
| Palp3/genital filaments | 142/115 | 0.432/0.463 | 36.1 |
| Palp5/coxite | 143/109 | 0.442/0.518 | 70 |
| Palp5/style | 143/109 | 0.901/1.011 | 54.8 |
| Palp5/paramere | 155/105 | 0.704/0.754 | 18.2 |
| Palp5/lateral lobe | 167/108 | 0.456/0.514 | 66.4 |
| Palp5/genital pump | 165/109 | 0.615/0.716 | 100.2 |
| Palp5/genital filaments | 163/106 | 0.42/0.456 | 23.6 |
| Mesonotum/coxite | 173/127 | 1.76/1.981 | 83 |
| Mesonotum/style | 173/126 | 3.553/3.847 | 142.5 |
| Mesonotum/paramere | 131/111 | 2.748/2.852 | 14.4 |
| Mesonotum/lateral lobe | 173/125 | 1.788/1.949 | 176.5 |
| Mesonotum/genital pump | 171/126 | 2.408/2.713 | 276.3 |
| Mesonotum/genital filaments | 169/122 | 1.642/1.742 | 37.3 |
| Wing length/coxite | 173/139 | 6.405/7.106 | 44.7 |
| Wing length/style | 173/138 | 12.951/13.869 | 68.3 |
| Wing length/paramere | 159/122 | 9.992/10403 | 16.6 |
| Wing length/lateral lobe | 171/137 | 6.488/7.042 | 110.8 |
| Wing length/genital pump | 171/138 | 8.699/9.821 | 213.7 |
| Wing length/genital filaments | 169/134 | 5.973/6.273 | 27.1 |
| a/coxite | 160/128 | 1.69/1.951 | 59.9 |
| a/style | 173/138 | 12.951/13.869 | 68.3 |
| a/paramere | 149/121 | 2.627/2.825 | 27.4 |
| a/lateral lobe | 158/126 | 1.712/1.923 | 90.4 |
| a/genital pump | 158/127 | 2.293/2.685 | 146.2 |
| a/genital filaments | 156/123 | 1.578/1.715 | 32.6 |
| b/coxite | 160/128 | 0.853/1.012 | 38.1 |
| b/style | 160/127 | 1.721/1.968 | 33.8 |
| b/paramere | 149/121 | 1.326/1.468 | 16.7 |
| b/lateral lobe | 158/126 | 0.864/0.994 | 39.4 |
| b/genital pump | 158/127 | 1.159/1.389 | 59.6 |
| b/genital filaments | 156/123 | 0.802/0.884 | 16.3 |
| Wing index/coxite | 159/128 | 0.006/0.008 | 37.6 |
| Wing index/style | 160/127 | 0.013/0.015 | 32.8 |
| Wing index/paramere | 149/121 | 0.01/0.011 | 14.5 |
| Wing index/ lateral lobe | 158/126 | 0.007/0.007 | 38.2 |
| Wing index/genital pump | 158/127 | 0.009/0.01 | 63.2 |
| Wing index/genital filaments | 156/123 | 0.006/0.007 | 16.8 |
| $R_5$/coxite | 137/81 | 4.101/4.384 | 40.3 |
| $R_5$/style | 137/81 | 8.415/9.103 | 73.7 |
| $R_5$/paramere | 125/77 | 6.471/6.778 | 13.7 |
| $R_5$/lateral lobe | 135/81 | 4.205/4.63 | 140 |
| $R_5$/genital pump | 137/81 | 5.636/6.48 | 215.4 |
| $R_5$/genital filaments | 136/80 | 3.886/4.171 | 37.3 |
| e/coxite | 161/127 | 2.111/2.415 | 64.8 |
| e/style | 161/126 | 4.267/4.708 | 97.5 |
| e/paramere | 150/120 | 3.286/3.499 | 29.8 |
| e/lateral lobe | 159/125 | 2.138/2.383 | 126.2 |
| e/genital pump | 159/126 | 2.868/3.328 | 200.1 |
| e/genital filaments | 157/122 | 1.97/2.127 | 42 |

$F_{critical,\ 0.1\%}$= 11- 11.1

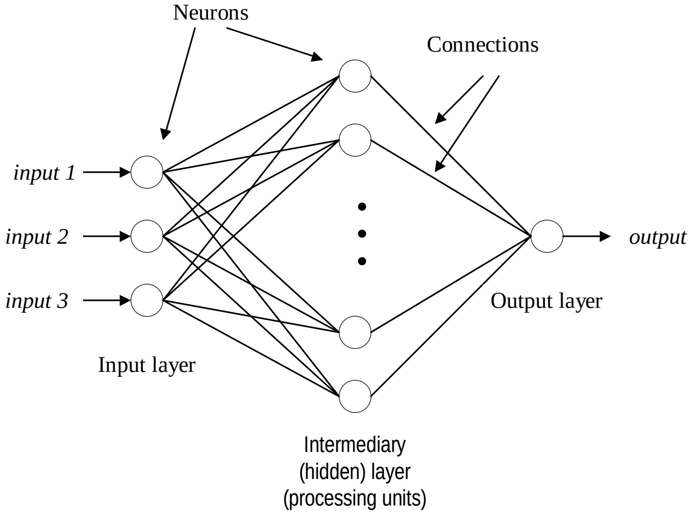
particular insect, was presented to an artificial neural network (ANN), using the software *QwikNet*, release 2.15. A group of 300 insects, randomly selected, was used to train the model and 58 were reserved for testing the model.

The input of the ANN consisted of 55 variables, 54 of which described the above referred ratios pertaining to the 358 insects, as explained above, and one referred to a number representing the origin of the individual. The last variable was included due to the assumed importance of the origin of the male and of its associated female in the identification (Marcondes et al. 1998a). Only one output was necessary, namely, the variable *species*, to which either 0 (*L. intermedia*) or 1 (*L. neivai*) was assigned. Because the insects have been divided into two classes (designated by the numbers 0 and 1), values smaller than 0.5 yielded by the ANN were considered as belonging to the category 0, and conversely, values greater than 0.5 as belonging to category 1. A registered version of software *QwikNet,* release 2.15, was employed to process the data. After experimenting with many alternatives and comparing the respective performance, the chosen topology of the net employed to process the data consisted of 56 input neurons (55 for the active variables and one for the *bias*), three hidden layers with 2, 5 and 2 neurons, respectively, and one output neuron. The mathematical algorithm utilized by the ANN for its training is designated by *RPROP* – resilient propagation. This is an adaptive learning rate method where weight updating is based only on the sign of the local gradients, not their magnitudes. Each weight $w_{ij}$ has its own step size or update value, which varies with time in the course of the process. A sigmoidal activation function was also employed. Figure illustrates the architecture of an ANN with one input layer, one output layer and an intermediary layer, also referred to as *hidden*.

ANN correctly classified 56 out of the 58 examples contained in the testing set (96.6%), according to the selected error margin (0.3). The classification of the examples belonging to the testing set was accomplished based solely on the input variables, so the ANN only used the targets to generate the pertaining statistics. In other words, the categories of the insects included in the testing data set could have been omitted, and the results would be identical.

Although there were many significant differences between the ratios of the morphological characters of the males of the two species, the great extent of overlapping of measurements regarding both species invalidated the direct use the ratios for legitimate specific identifications.

On the other hand, the ANN yielded very reliable identifications. This technique comes from the field of computational intelligence. One of its main applications is the classification of objects, having a set of examples as a basis, with the correct categories previously defined. To perform a classification task, an ANN relies on mathematical algorithms that pursue the establishment of an association between the input variables and the outputs. This feat is accomplished by adjusting internal parameters – weights – that link the nodes, also called artificial neurons. In this way, a mapping [inputs ® outputs] is performed. Different from traditional statistical methods, where some function underlying the data is sought, ANN's make use of an *implicit* mapping, which results from the



Schematic representation of an artificial neural network with three inputs and one output connectionist character of this paradigm (Kasabov 1996). The ANN technique is growing as a very useful and efficient tool to deal with problems where a reasonable set of data is available, but where a proper analytical or statistical model capable of associating inputs and outputs cannot be found. The mathematical details of the algorithm and the characteristics of the ANN's are beyond the scope of this work.

The use of ANN should be tested in other groups of species in which the distinction of insects of one sex is difficult, such as pertaining to *L. shannoni*/*L. abonnenci*, *L. wellcomei*/*L. complexa*, *Simulium damnosum* and *Anopheles gambiae* species complexes.

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