

Cytological Analyses Reveal Variations in Nuclear Content Along the Urediniosporic Infection Cycle of *Hemileia vastatrix* and other Rust Fungi

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The recent completion of genome sequencing for some rust fungi has contributed to suggest a link between biotrophic specialization and genome size expansion. The measurement of genome sizes for a selection of rust fungi has revealed some of the largest genomes among fungi, with nine rust species with haploid genomes between 300 and 780 Mbp. In particular, *Uromyces appendiculatus*, *Phakopsora pachyrhizi*, *U. transversalis*, and *Hemileia vastatrix* genomes were measured as 652, 716, 746, and 772 Mbp, respectively. Moreover, flow cytometric analysis of *H. vastatrix*-infected coffee (*Coffea* spp.) leaf samples consistently yielded profiles compatible with the occurrence of three different nuclear contents (n, 2n, and 4n). The n and 2n populations contained relatively similar numbers of nuclei, whereas the 4n population clearly represented a minority. Pre-sporulating and sporulating (urediniosporic) coffee leaf infections presented these three nuclei populations. The n population was absent from both resting urediniospores and germinating urediniospores but reappeared in appressoria. These findings are compatible with the occurrence of

karyogamy and meiosis prior to sporulation, although parasexuality phenomena cannot be ruled out. In fact, although several orthologs of meiosis-specific genes are present in the *H. vastatrix* genome, only one of these was identified in *H. vastatrix* expressed sequence tag (EST) libraries obtained from germinating urediniospores, appressoria, and pre-sporulating infected leaves. Some karyogamy-related genes were identified both in the genome and in the EST libraries. Moreover, the occurrence of three different nuclear contents was also verified in infected host samples (at the urediniosporic stage, just before sporulation) for at least *Phakopsora pachyrhizi*, *Puccinia pelargonii-zonalis*, *U. appendiculatus*, and *U. fabae*, in contrast with the absence of the n population in resting and germinating urediniospores. This finding suggests that this phenomenon may be common across the Pucciniales.

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