Accelerated immunosenescence in preindustrial twin mothers

Samuli Helle*†, Virpi Lummaa‡, and Jukka Jokela§

*Section of Ecology, Department of Biology, University of Turku, FIN-20014, Turku, Finland; ‡Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom; and §Department of Biology, University of Oulu, POB 3000, FIN-90014, Oulu, Finland

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Life-history theory predicts a tradeoff between reproductive effort and lifespan. It has been suggested that this tradeoff is a result of reproductive costs accelerating senescence of the immune system, leading to earlier death. Longevity costs of reproduction are suggested for some human populations, but whether high reproductive effort leads to impaired immune function is unknown. We examined how reproductive effort affected postreproductive survival and the probability of dying of an infectious disease in women born in preindustrial Finland between 1702 and 1859. We found that mothers delivering twins had reduced postreproductive survival after age 65. This effect arose because mothers of twins had a higher probability of succumbing to an infectious disease (mainly tuberculosis) than mothers delivering singletons. The risk among mothers of twins of dying of an infectious disease was further elevated if mothers had started reproducing early. In contrast, neither female postreproductive survival nor the risk of succumbing to an infectious disease was influenced by the total number of offspring produced. Our results provide evidence of a long-term survival cost of twinning in humans and indicate that the mechanism mediating this cost might have been accelerated immunosenescence.

immune function | cost of reproduction | longevity | reproductive effort | tuberculosis

Life-history theory states that the negative relationship between reproductive effort and survival is the underlying evolutionary explanation for senescence (1–5). This tradeoff is believed to be mediated through allocation of limited resources between competing functions of reproduction and somatic maintenance and repair (5), but the underlying physiological mechanisms still remain elusive. One mechanism that has attracted attention recently is that high reproductive effort may impair immune function against pathogens (reviewed in refs. 6–9), and this may be true also in the long term (10). Maintenance of effective immune function may also involve costs (7, 8, 11, 12), which, in turn, may suppress the ability of an individual to allocate resources to reproduction, especially in environments where the risk of infection is high (13).

The evolution of senescence through a tradeoff between reproduction and survival is established in many model organisms (1, 2, 14). However, whether human long-term survival is influenced by the costs of reproduction remains controversial. This controversy may, in part, be due to confounding variables, such as individual quality (i.e., frailty) (15–17) and the lack of adequate measures of reproductive effort relevant to long-term survival (18, 19). In addition, it is currently unclear how the potential reproductive costs might be mediated on longevity in humans. For example, whereas there is evidence from contemporary human populations that high reproductive effort might increase the incidence of cardiovascular diseases in females (20), childbearing may also protect females from ovarian and pancreatic cancers (21, 22). Although the relationship between longevity and immunosenescence is well described in the medical literature (23, 24), the potential association between accelerated immunosenescence and high reproductive effort has not been investigated.

The most significant ways in which females can increase their reproductive effort is through giving birth to many offspring and through delivery of multiples, usually twins. That twin deliveries may also pose an elevated cost to mothers is supported by the findings that, after twin births, mothers have longer birth intervals to subsequent deliveries and are more likely to terminate reproduction completely (especially after male–male twins) (25). Although twin births are known to increase the risk of maternal mortality at childbirth (26, 27), their long-term consequences on female survival are unclear.

Here, we aim to test the hypothesis that increased reproductive effort expressed as high total number of offspring born and twin deliveries leads to reduced female postreproductive survival through accelerated immunosenescence in humans. To test this prediction, we first compare the postreproductive survival of 18th- and 19th-century Finnish women who produced at least one set of twins versus those who produced only singletons. We also investigate whether the number of offspring born was related to female long-term survival, controlling for other measures of maternal reproductive effort (i.e., ages at first and last reproduction) and potentially confounding effects of among-individual variation in wealth, as well as temporal and spatial variation in the associations studied. Second, we examine whether such estimates of reproductive effort were related to the postreproductive mothers' likelihood of dying of an infectious disease, as predicted by the immunosenescence hypothesis.

Methods

Demographic Data. Because the long-term consequences of reproductive effort on longevity and immunosenescence should be most evident in human populations living before industrialization and without effective birth control methods and advancements of modern medical care, we used demographic data from preindustrial Finnish mothers (born 1702–1859) obtained from five parish registers (Hiittinen, Kustavi, Pulkkila, Rymättylä, and Ikaalinen) kept by the Lutheran Church (28, 29). During the study period, these populations depended on farming and fishing for their livelihood (30). These demographic data are particularly suitable for examination of evolutionary relationships between reproduction and longevity (1, 2) because earlier studies have shown that in these populations both twinning and long postreproductive lifespan increased female fitness (30, 31).

We searched the parish registers for all women who produced twins (n = 213) in the studied populations between 1743 and 1889. These mothers of twins (born between 1702 and 1845) were contrasted with mothers who produced only singletons during their lifetime (born between 1710 and 1859). This sample of mothers of singletons (n = 594) consists of two groups of

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Abbreviation: C.I., confidence interval.

†To whom correspondence should be addressed. E-mail: samuli.helle@utu.fi.

Table 1. Female life-history traits (mean ± SD) in postreproductive mothers who produced twins and two groups of mothers who produced only singletons in preindustrial Finnish populations studied (1702–1859)

Mothers of	Lifespan, years	No. of offspring born	Age at first reproduction, years	Age at last reproduction, years
Twins	66.51 ± 9.92	7.70 ± 2.73	25.72 ± 4.48	40.37 ± 4.05
Singletons				
Matched sample	67.11 ± 11.20	6.88 ± 2.78	24.79 ± 4.32	40.71 ± 4.07
Random sample	67.49 ± 11.46	5.22 ± 2.77	26.64 ± 5.29	39.09 ± 4.93

mothers. The members of the first group of mothers ($n = 242$) were originally matched with the mothers of twins by the parish in which they were born, the year they were born, and the number of previous births (30). The second group of mothers ($n = 352$) was chosen randomly among those mothers who delivered only singletons in the study parishes during the study period. We did not analyze the data by using a matched-paired design to allow the inclusion of the social class of the mothers (not considered when choosing the first group of singleton mothers) and maximization of our sample size. Pooling all of the data on mothers of singletons together was justified, because the life-history traits of matched and randomly selected mothers of singletons deviated <1 SD from each other (see Table 1). Population twinning rates varied among the populations (30), being highest (2.13%) in the archipelago areas (Hiittinen, Kustavi, and Rymättylä).

For all of these women, complete life histories were known, including ages at first and last reproduction and total number of offspring born (Table 1). Information on the occupation of each woman's husband (e.g., priest, landowner, tenant farmer, servant, etc.) allowed us to rank the social class of each family, which is a correlate of family resources (29), and hence an important potential confounding variable in studies concerning phenotypic tradeoffs (17). Likewise, because immunological tradeoffs might be manifested, particularly in poor environmental settings (32), we divided the study populations into two geographical areas with varying ecological conditions (archipelago versus inland areas) (30) and recorded birth cohort for each woman to control for confounding geographic and temporal variation in survival and reproduction.

To focus on the long-term survival effects of reproductive effort, we included only postreproductive women in our analysis. A mother was regarded as postreproductive if she lived beyond the age at which 99% of all mothers in her population of birth had delivered their last child. These ages were 49, 46, 47, 47, and 49 years for our five study populations (Hiittinen, Kustavi, Pulkkila, Rymättylä, and Ikaalinen), respectively. This restriction gave us a sample size of 625 women, of which 167 mothers gave birth to at least one set of twins and the remaining 458 mothers delivered singletons only. The inclusion of postreproductive mothers only ensured the avoidance of spurious positive correlations between reproductive performance and survival arising from the fact that women dying young also had fewer opportunities to bear offspring.

We were able to record the cause of death for almost 82% ($n = 514$) of these postreproductive Finnish mothers (90% of twin and 80% of singleton mothers) (Table 2). This allowed us to study the relationship between reproductive effort and the likelihood of dying of an infectious disease (33). Mothers were divided into categories based on whether they died of an infectious disease or of some other common stated cause of death (Table 2).

Statistical Analyses. We used piecewise Cox regression to examine how reproductive effort was associated with female postreproductive survival (34, 35). We included multiple delivery (whether a mother delivered twins or not), total number of offspring born, age at first and last reproduction, social class (poor or rich), geographical area (archipelago or inland), and maternal birth cohort as independent variables in the survival analysis. Because the survival curves of mothers of twins and singletons crossed at age 65, suggesting nonproportional hazards with age (see Fig. 1*A*), we estimated hazard ratios separately before and after age 65 (34) for the effect of twin delivery. Two- and three-way interactions among geographical region, social class, and estimates of reproductive effort were first added into the model. If these terms, as well as the main terms, were not statistically significant ($\alpha < 0.05$) when the log-likelihood ratio test was used for comparing nested models (34), they were omitted from the final model. Assumption of proportional hazards was checked by including time-dependent covariates of explanatory variables in the model (34, 35). No evidence of nonproportionality of hazards was found. For mothers of twins, we further assessed whether the sex ratio of twins or mother's age at twin delivery affected her postreproductive survival, because female lifespan may depend on the offspring sex ratio and on the timing of births (18, 19).

The probability of dying of an infectious disease as a function of twin births, the number of offspring born, and other recorded parameters of reproductive effort were studied by using logistic regression (36). All two- and three-way interactions among measures of reproductive effort, social class, and geographical

Table 2. Summary of the causes of death of postreproductive mothers of twins and singletons in preindustrial Finnish populations studied

Cause of death	No. (%) of mothers	
	Mothers of twins	Mothers of singletons
Infectious diseases		
Tuberculosis	28 (18.7)	44 (12.1)
Pneumonia	10 (6.7)	11 (3.0)
Typhoid fever	5 (3.3)	10 (2.8)
Dysentery	5 (3.3)	4 (1.1)
Endemic malaria	5 (3.3)	13 (3.5)
Spotted fever	4 (2.7)	5 (1.4)
Leprosy	1 (0.7)	2 (0.6)
Unspecific fevers	9 (6.0)	14 (3.9)
Diarrhea	1 (0.7)	2 (0.6)
Scarlet fever	0	1 (0.3)
Stomach disease	0	2 (0.6)
Total	68 (45.3)	108 (29.7)
Other causes		
Weakness and old age	45 (30.0)	128 (35.2)
Cardiovascular diseases	4 (2.7)	30 (8.2)
Cancers	0	6 (1.7)
Unknown reasons	10 (6.7)	23 (6.3)
Other causes of death	23 (15.3)	69 (19.0)
Total	82 (54.7)	256 (70.3)
Total	150	364

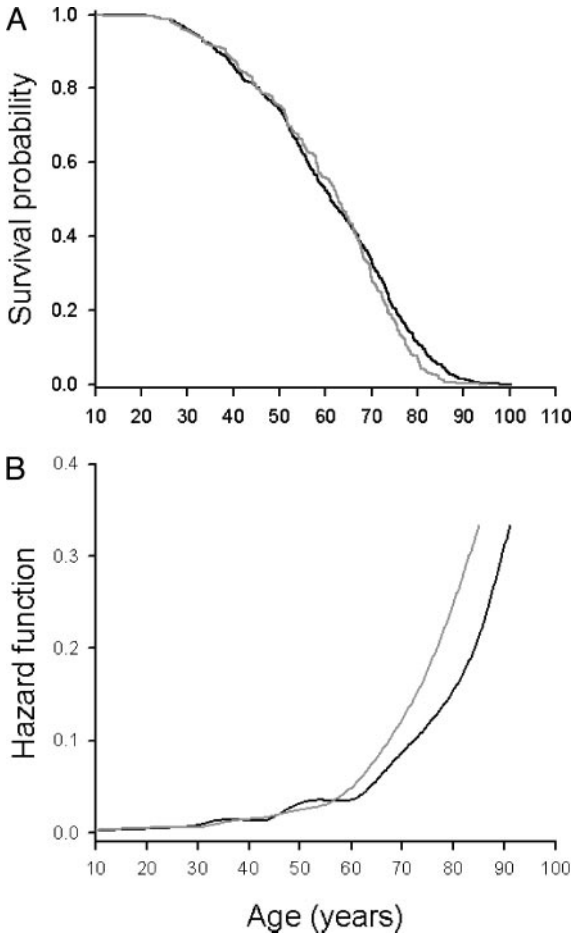

Fig. 1. Kaplan-Meier survival (*A*) and hazard function (*B*) plots of reproducing preindustrial Finnish mothers according to whether they delivered at least one set of twins (gray line) or only singletons (black line) during their lifetime. The hazard rates are estimated for 6-year intervals. Note that the survival analysis was conducted by including only postmenopausal women (see *Methods*).

area were added in the initial model and, if statistically significant at the α level of 0.05, included in the final model. Finally, we examined the potential effects of twin sex ratio and maternal age at twin delivery on the likelihood of dying of an infectious disease among mothers of twins.

Results

Reproductive Effort and Postreproductive Survival. Female postreproductive survival varied only according to whether the mother delivered twins or not. Mothers of twins had a 39% higher hazard of death compared with mothers of singletons after age 65 (Table 3; Fig. 1 *A* and *B*). Total number of offspring born, age at first and last reproduction, social class, birth cohort, and geographical area were not significantly related to postreproductive survival among these mothers (Table 3). Moreover, the postreproductive survival of mothers of twins was independent of the sex ratio of twins ($\chi^2_2 = 1.845$, $P = 0.40$) and the age of the mother when she delivered twins [hazard ratio = 0.978; 95% confidence interval (C.I.) = 0.950–1.008; $\chi^2_1 = 2.067$, $P = 0.15$]. Note that these results are true for mothers who lived beyond their reproductive years. However, there was no difference between mothers of twins and mothers of singletons in the likelihood of reaching postreproductive age (odds ratio = 1.012; 95% C.I. = 0.684–1.498; $\chi^2_1 = 0.04$; $P = 0.95$, adjusting for birth cohort). The age-dependent survival of mothers of twins and mothers of singletons remained similar if we also included mothers who died during their reproductive years in the analysis [age $\leq$ 65: hazard ratio = 1.007 (95% C.I. = 0.815–1.243), $\chi^2_1 = 0.003$, $P = 0.96$; age $>$ 65: hazard ratio = 1.553 (95% C.I. = 1.221–1.974), $\chi^2_1 = 12.05$, $P = 0.0005$, adjusting for age at last reproduction, geographical area, and social class].

Reproductive Effort and Mortality from Infectious Disease. Postreproductive mothers of twins had nearly a six times higher risk of dying of an infectious disease compared with mothers of singletons after adjusting for the effects of age at first reproduction and the interaction between age at first reproduction and multiple delivery (Table 4) [unadjusted odds ratio for the effect of multiple delivery was 1.966 (95% C.I. = 1.328–2.910); see Table 2]. Total number of offspring born, age at last reproduction, geographical area, birth cohort, and social class did not have a significant effect on the probability of succumbing to an infectious disease (Table 4). We also detected a significant interaction between age at first reproduction and multiple delivery (Table 4). When we reran our analysis separately for mothers of twins and singletons, we found that late age at first reproduction reduced the risk of dying of an infectious disease by almost 9% per year among mothers of twins [odds ratio = 0.915 (95% C.I. = 0.847–0.990), $\chi^2_1 = 4.90$, $P = 0.027$] (Fig. 2) but had no effect on that risk among mothers of singletons [odds ratio = 1.024 (95% C.I. = 0.980–1.070), $\chi^2_1 = 1.13$, $P = 0.29$] (Fig. 2). Note that mothers of singletons seemed to have a higher risk of dying of an infectious disease than mothers of twins if they started reproducing after age 32 (Fig. 2). However, this result is

Table 3. Piecewise Cox proportional hazard model for the effects of recorded reproductive parameters on the postreproductive survival of historical Finnish mothers

Effect	−2LL*	df	χ^2	*P*	Hazard ratio (95% C.I.)†
Null model	6725.74				
Multiple delivery (>65)	**6718.61**	**1**	**7.127**	**0.0076**	**1.394 (1.099–1.768)**
Multiple delivery (≤65)	**6718.58**	**1**	**0.033**	**0.86**	**0.975 (0.745–1.278)**
Age at last reproduction	6715.40	1	3.184	0.074	0.984 (0.968–1.001)
Birth cohort	6706.56	4	8.839	0.065	—‡
Geographical area	6705.06	1	1.499	0.22	1.128 (0.932–1.365)
Social class	6704.53	1	0.533	0.47	1.069 (0.893–1.278)
Number of offspring born	6704.43	1	0.096	0.76	0.994 (0.960–1.030)
Age at first reproduction	6704.40	1	0.029	0.87	0.998 (0.972–1.024)

Statistically significant terms included in the minimal model are given in bold.
*Log likelihood (× −2) of the model.
†C.I. denotes 95% confidence intervals of hazard ratios.
‡Hazard ratios omitted because of the large number of categories of birth cohort.

Table 4. Female reproductive parameters and the probability of dying of an infectious disease among postreproductive Finnish mothers studied

Effect	df	χ^2	*P*	Odds ratio (95% C.I.)
Multiple delivery (MP)	**1**	**8.93**	**0.003**	**5.960 (1.849–19.215)**
Age at first reproduction (AFR)	**1**	**1.99**	**0.16**	**0.968 (0.926–1.013)**
MP × AFR	**1**	**6.01**	**0.014**	**0.946 (0.904–0.989)**
Social class	1	1.12	0.29	0.798 (0.526–1.211)
Number of offspring born	1	1.02	0.31	0.958 (0.880–1.042)
Birth cohort	4	2.29	0.68	—*
Geographical area	1	0.12	0.73	0.913 (0.545–1.529)
Age at last reproduction	1	0.009	0.92	0.997 (0.939–1.059)

Statistically significant terms included in the minimal model are given in bold.
*Odds ratios omitted because of the large number of categories of birth cohort.

of small impact and unlikely to change our conclusions, because >90% of mothers of singletons and twins had already begun reproducing by that age. The increased risk among mothers of twins of dying of an infectious disease remained evident after restricting the analysis to include only women surviving beyond age 65 [odds ratio = 2.395 (95% C.I. = 1.281–4.478), $\chi^2_1 = 7.48$, $P = 0.006$]. In addition, among mothers of twins, neither twin sex ratio ($\chi^2_2 = 1.85$, $P = 0.40$, adjusting for birth cohort) nor maternal age at twin delivery [odds ratio = 0.952 (95% C.I. = 0.885–1.024), $\chi^2_1 = 1.74$, $P = 0.19$, adjusting for cohort] influenced a mother's risk of dying of an infectious disease.

In particular, mothers of twins had a 77% higher risk of dying of tuberculosis than mothers of singletons [odds ratio = 1.774 (95% C.I. = 1.052–2.994), $\chi^2_1 = 4.61$, $P = 0.032$, adjusting for geographical area], which was the most common infectious disease causing postreproductive mortality in the populations studied during the study period (Table 2). A similar but nonsignificant trend was found for the likelihood of dying of pneumonia [odds ratio = 2.100 (95% C.I. = 0.864–5.100), $\chi^2_1 = 2.68$, $P = 0.10$, adjusting for geographical area].

To exclude the possibility that these effects arose because mothers of singletons were more likely to die of an infectious disease before reaching postreproductive years, we repeated our analysis, including only mothers who died during the reproductive years but not at child birth ($n = 128$, of which 25 mothers delivered twins and 103 mothers delivered singletons). We found no difference between the reproductive-aged mothers of twins and singletons in their likelihood of succumbing to an infectious disease [odds ratio = 1.324 (95% C.I. = 0.505–3.469), $\chi^2_1 = 0.33$, $P = 0.57$]. This finding indicates that twin deliveries were only related to increased mortality from infectious diseases during the postreproductive, but not during the reproductive, years.

Fig. 2. Predicted probability of dying of an infectious disease as a function of age at first reproduction for mothers of twins (gray line) and singletons (black line).

Discussion

Our results show that delivering twins, irrespective of the age of the mother at delivery or the sex ratio of the twins, resulted in reduced postreproductive survival after age 65 in preindustrial Finnish women. Our findings further suggest that this might have been due to accelerated immunosenescence in mothers of twins, because mothers of twins were more likely to die of an infectious disease (mainly tuberculosis) than mothers of singletons. Young age at first reproduction seemed to further increase the risk of succumbing to an infectious disease, but only among mothers of twins. This finding indicates that early reproductive effort may also have been relevant for the expression of immunosenescence when mothers produced twins.

These findings reveal a long-term survival cost of twinning, which was likely brought about by the increased risk of dying of an infectious disease. As mothers of twins did not show higher infection-related mortality during their reproductive years compared with mothers of singletons, we can exclude the alternative explanation that twinning was more frequent among those women who expressed lower levels of immune defense generally. However, because the causality between twinning and impaired immune function cannot be unequivocally determined, we cannot definitely rule out the explanation that twin births occurred among females with higher overall rates of senescence. Nevertheless, this explanation would appear unlikely, because mothers of twins did not suffer from higher mortality during their reproductive years (Fig. 1 *A* and *B*) and were not less likely to reach postreproductive ages. In addition, our results are not likely to be confounded by social or environmental factors, because our analyses controlled for the social class of mothers and temporal and spatial differences in living conditions.

The likelihood of succumbing to an infectious disease seemed to be further affected by age at first reproduction in mothers of twins. This result highlights the significance of reproductive timing on the deterioration of the female postreproductive immune system and may be of evolutionary importance because the early start of a reproductive career is shown to be one of the most important components of female fitness in both historical and contemporary human populations (37, 38). Moreover, this finding also encourages a search for potential mechanistic links between early reproduction and the development of different age-specific infectious diseases (39) and their association to twin deliveries.

In 18th- and 19th-century Finland, as elsewhere in Europe, tuberculosis was common: many were infected with the disease early in life and carried it dormant for long periods of time before developing acute symptoms as their immune systems became compromised (40, 41). It is therefore not surprising that the higher mortality of mothers of twins was mainly due to reduced resistance to tuberculosis, and, to a lesser extent, pneumonia, both of which are known to be the leading infectious causes of morbidity and mortality in the elderly, even in modern day societies (41, 42). Although mortality from tuberculosis within a year of giving birth has been previously found to be elevated in historical populations (33), our results provide evidence that such effects may also have persisted in the long run if the delivery produced twins.

However, examining the causes of death in detail (Table 2) reveals that those postreproductive mothers who delivered only singletons were over three times more likely to die of cardiovascular diseases than mothers of twins [odds ratio = 3.278 (95% C.I. = 1.134–9.475), $\chi^2_1 = 4.81$, $P = 0.028$]. One may speculate whether this is due to some "protective" effects of twinning or is simply a reflection of the longer lifespan and lower incidence of disease-related mortality among mothers of singletons. Such protective effects of twinning could be hypothesized to be brought about by higher levels of estrogens (by means of elevated follicle-stimulating hormone levels) among reproductive-age mothers who delivered twins, which may in turn reduce the risk of cardiovascular diseases later in life (reviewed in refs. 43, 44). If mothers of twins turn out to have lower mortality from cardiovascular diseases also in contemporary populations, this might have medical significance, because cardiovascular diseases are a major cause of death in the developed world.

If twinning led to a reduced postreproductive lifespan in environments of high infection risk, we may have identified a new cost of twinning that selection could have operated on during human brood-size evolution. We have previously shown that, historically, twin births often enhanced female fitness in humans (25, 30, 45), and recently Lahdenperä *et al.* (31) reported positive selection on female postreproductive lifespan in the same populations of Finns. If long postreproductive lifespan indeed brought fitness benefits to the females, twinning may have been selected against in historical human populations where the risk of infection was high. More generally, that tuberculosis and other infectious diseases have posed constant threats to human survival for centuries might, in part, have reduced the spread of genes with a propensity for causing twinning. In addition, the result that reproductive effort may lead to earlier immunosenescence suggests that the interaction between reproductive life-history traits, such as twinning, age at first reproduction, and lifespan, and complex physiological pathways may be much more fundamental and potentially long term than earlier thought.

In contrast, our findings do not provide support for the idea that impaired female immune function and reduced postreproductive lifespan are consequences of high total investment (often measured as a total number of offspring produced) in reproduction (6–9). In light of this, we find it intriguing that the cumulative effects of singleton births seem to be weaker than a single event of twin birth with respect to maternal immunosenescence and lifespan. It is currently unclear what the particular physiological mechanism mediating such an effect could be. One possibility is that twin births may be related to elevated maternal stress levels (46), which might have had a direct negative bearing on maternal immunocompetence and thus on their long-term survival (47). Finally, we emphasize that these findings concern women that lived before modern medical assistance. To speculate whether processes reported in this study might still be relevant, we encourage challenging our findings with data sets collected from modern human populations.

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